Chapter 8
Trichoderma: Biocontrol Agents for Promoting Plant Growth and Soil Health

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8.1 Introduction

Trichoderma is a saprotrophic fungus which largely can be found in environments such as forest soil, roots and leaves. This fungus has been declared as soil fungi due to its significant for their fast growth. They exhibited high capacity to utilize different types of complex substrates and can act as a strong resistances towards different kind of toxic chemicals. Therefore, *Trichoderma* species is very abundance on decaying wood. This is mainly because of the heterotropic interactions such as decomposition and opportunistic endophytism. It can be found in all type of soils which includes from forest, salt marsh, agricultural even in desert soils. In addition to that, *Trichoderma* has been used as an efficient biocontrol agent against the phytopathogens (Sharma et al. 2019). The main mechanisms for the biocontrol process in this type of fungi have been assumed due to antibiosis, mycoparasitism and competition for space and resources (Bissett et al. 2015).

This fungus evolved many mechanisms which contribute for the improvement of the plant resistance towards diseases, the plant's growth as well as its productivity (Rastegari et al. 2020; Yadav et al. 2020d, 2019b). According to Bissett et al. (2015), out of 260 species, around 35 established species was mainly discovered for its economic importance mainly due to its capability of various enzyme productions or to be used as biocontrol agents. Global interest was given to researches related to *Trichoderma* fungus thanks to its applications in the field of agricultural and biotechnology (Verma et al. 2017a).

Trichoderma has been thought to be associated with plants as epiphytes and endophytes. Epiphytes are beneficial microflora that can be found at the aerial parts of the plants that can be either harmful or beneficial towards the plant (Yadav et al. 2020b, c). The word endo derived from the Greek words which carry the meaning of within and "phyte" stands for plant. In another word, endophytes are the colonization which takes place inside the plants by any fungus or bacteria (Suman et al. 2016; Verma et al. 2017a; Yadav et al. 2020a). There are few reports supporting the fact that *Trichoderma* species found as endophytes of the plants. One of the well-known examples is the presence of *Trichoderma* in banana tissues (Wang et al. 2000). According to the Pocasangre et al. (2000) study on the distribution of endophytic fungi from banana was conducted which is mainly about isolating the fungus from the central cylinder. Besides that, Sikora et al. (2008) did survey on isolating endorhiza of banana and use it for biocontrol of nematodes.

8.2 Methodology of Studying *Trichoderma* Biodiversity

Most of the species identification for *Trichoderma* species has been performed according to their morphological characteristics. In order to carry out this, solid media with different components which are targeted for conidium production and its branching observation were used for the culture characteristics as well as for the morphological studies (Hermosa et al. 2000). Most of the times, the morphological studies which aimed for conidiosphores structure identification can be done by referring to the taxonomic keys and the information provided by the literature studies. However, without having any professional expertise this practice may lead to incorrect conclusion. In order to overcome this issue for better species identification, biochemical and molecular methods are highly recommended (Photita et al. 2001).

One of the useful tools which used for better species identification and give data for the ecological purpose of the species is metabolic profiling technique which also provides the possibilities of the quantitative measurement of the growth and consumption of different carbon and nitrogen sources. According to Kredics et al. (2011), the cellulose-acetate electrophoresis based isoenyzme analysis with using glucose-6-phosphate dehydrogenase, glucose-6-phosphate isomerase, 6-phosphogluconate dehydrogenase, peptidases A, B and D and phoshoglucomutase enzymes was used for isolation and identification of *Trichoderma* strains which derived from some clinical samples and wheat fields. On the other hand, another alternative for the biochemical technique was suggested by Neuhof et al. (2007), where the identification process was mainly based on intact-cell mass spectrometry. This method will help to identify hydrophobins in the mycelia as well as spores of the strains. The patterns of the hydrophobin reflect the characteristic of the species that being isolate and this method can also give a rapid and direct identification of class II hydrophobins (Hanada et al. 2008).

When it comes to the molecular methods, methods like the DNA-fingerprinting, the sequence analysis of ribosomal internal transcribed spacer (ITS) region and the segments from genes encoding for the translation elongation factor 1-alpha (*tef1*), endochitinase (*chi18-5*, formerly known as *ech42*), RNA polymerase II subunit (*rpb2*) and calmodulin (*cal1*) is the suitable method for giving the accurate species identification for *Trichoderma* strains (Druzhinina et al. 2008). The *Trich*OKEY which is also known as ITS-based online barcoding program becomes another useful tool for the species identification. For the analysis of *tef1*, ITS and *rpb2* sequences the online programme known as *Tricho*BLAST and its updated version named *Tricho*MARK can be used. As an example, according to Nagy et al. (2007), by using the online barcoding programme name *Tricho*CHIT, any potential chitinase producing strain such as *Trichoderma harzianum* was used. Moreover, species-specific primers in polymerase chain reaction can be used for a quick and precise diagnosis. According to Kredics et al. (2011), this method allows for a fast and specific identification of *Trichoderma pleurotum* and *Trichoderma pleuroticola.* Both the strain is known as agent that causes the formation of green mould in the global production of *Pleurotus ostreatus* within the growing substrate and without any requirement of cultivation time (Xia et al. 2011a, b).

The studies related to *Trichoderma* biodiversity mainly based on the standard culture approach which contains the collection of samples, the isolation of *Trichoderma* strains on selective media, however when this method is being applied, some strains can be easily identified and there are some which is even harder to do the isolation procedure. The diversity that had been detected by using this method does not reflect the accurate diversity of the genus from the tested habitat. The metagenomic method provides the best solution to overcome this problem as it examining the habitat without doing any isolation or culturing of the strains. When the first attempt is done on biodiversity detection using this metagenomic approach by Hagn et al. (2007) the primers for *Trichoderma* specific were created for the ITSI fragment of the rRNA gene cluster. However, using this approach, only around 12 species have been identified as this ITSI alone is could not identify all the species because certain strains share the same allele (Samuels et al. 2006).

To overcome these issues, Meincke et al. (2010) created the primer pair which is specific for *Trichoderma* for diversity analysis purpose. This specific primer help to amplify around 650 bp fragment of the ITS region which is suitable for identification by *TrichO*KEY and *Tricho*BLAST. The very first PCR-amplification was performed by having fungal specific forward primer and *Trichoderma*-specific reverse primer. For the second reaction, the *Trichoderma*-specific forward and reverse primers are used together. The ITS amplicons help to denature the gradient gel electrophoresis or help to clone to the pGEM-T easy vector. The currently developed primer system was applied on *Trichoderma* communities in the rhizosphere of potatoes. But this method could not detect the several species because reverse primer is located 30 bp upstream of the last genus-specific *Trich*OKEY hallmark (Nawaz et al. 2018).

However, in a recent study conducted by Nawaz et al. (2018), six reverse primers were designed and their specificity and selectivity were tested. Along with the forward primer ITS5 this reverse primer can help to amplify the whole diagnostic region of ITS1. The six separated PCR amplifications from the tested soil that contain same forward and one reverse primers were combined, purified and subcloned to pGEM-T. The sequences of the individual clones were determined and analysed with *Trich*OKEY 2.0 and *Tricho*BLAST. Atanasova et al. (2010) applied this strategy to determine the diversity of the strain in samples.

8.3 *Trichoderma* Diversity in Different Habitats

8.3.1 Natural Soils, Decaying Wood and Plant Material

Danielson and Davey (1973) found out that at the south part of the USA and at the state of Washington, *Trichoderma* can be found at variety of forest soil. Some of the strains like *Trichoderma polysporum* and *Trichoderma viride* were located near the strictly cool temperature regions. *Trichoderma harzianum* was isolated from warm climate area, while *Trichoderma hamatum* and *Trichoderma pseudokoningii* were dominantly growing at area with excessive moisture. Besides that, the isolation, morphological characterization as well as full identification of strains like *Trichoderma aureoviride*, *T. harzianum*, *T. koningii*, *T. longibrachiatum* and *T. viride* from area like the wood of the oak, cork wood and dead wood of the apple twigs have been studied (Vasanthakumari and Shivanna 2011).

The broad studies on the biodiversity of *Trichoderma* were conducted in North America and at some region in Europe. The isolated *Trichoderma* strains were analysed for their function to degrade the organochlorine xenobiotics but as mentioned earlier, the results from this method are difficult to be evaluated. This is mainly due to the fact that there is no available of molecular tools for the identification (Vasanthakumari and Shivanna 2011). According to Zhang et al. (2005), around 135 isolates of *Trichoderma* were obtained from four regions of China which are from Hebei, the northmost side, followed by Zheji-ang, the south-east side, Yunnan, west part and from the Himalayan part as well. The result from this study indicates from among the North to South part, the Northern China finalized as potential centre for origin of the haplotype of *Trichoderma harzianum* (Vasanthakumari and Shivanna 2011).

One the other hand, Abd-Elsalam et al. (2010) isolated the fungi strain from the soil of protected area in Saudi Arabia and the identification process were carried out by suing M13-microsatelite PCR and ITS barcoding. Results from the investigation reveal that *Trichoderma harzianum* and *Trichoderma longibrachiatum* are present. The biodiversity study of *Trichoderma* from the European river floodplain habitat near the Danube national park, which was originally known for its riparian forest area in Vienna, Austria was carried out mainly based on the morphological examinations, sequence analysis of the ITS region and fragment of the tef1 gene as RAPD analysis (Zhang et al. 2005). Plant litter associated fungi was examined from the spring of the sheep herd in the western of Norway and isolated wide variety of fungi which includes two *Trichoderma* strains. However, the authors failed to determine the species by NCBI BLAST for its ITS sequences. But by using *Trich*OKEY the isolated named as *Trichoderma hamatum* while other were identified as *Trichoderma koningiopsis* (Sun et al. 2012).

A wide-scale survey was done over 14 European countries which have temperate climate in order to learn about the biodiversity of *Trichoderma* by testing their morphology, ITS and tef1 sequences. Around 75 species were detected from European countries. By doing ITS barcoding-based study, some of the strains are found from soil and decaying wood. Then taxon-specific metagenomic method was used by Friedl and Druzhinina (2012), in order to determine the *Trichoderma* biodiversity in situ in soil samples from aspen and beech forests along the Danube floodplain. The obtained result shows that not only small number of *Trichoderma* species were capable of forming teleomorph but suggested that the biodiversity of the genus is higher on and above the small layer than inside the soil. Some of the strains associated with the host fungi or trees were found but the major of the species suggested to be necrotrophic on diverse fungi on wood and tree bark (Samuels et al. 2012b).

8.3.2 Endophytes

Living plants or endophytes can be defined as organisms which live within the plant cells. They established a relationship with the plant that varies from symbiotic to pathogenic (Bailey et al. 2006). There are certain types of *Trichoderma* strains which are capable of colonizing the root part of the plant and continue to have a symbiotic relationship with that particular plant. There has been a lot of investigation being carried out in order to prove that *Trichoderma* strains can develop endophytic relationship within the inner tissue part of the plant (Samuels et al. 2012a).

Theobroma cacao which commonly known as cocoa plant was become the first target to carry out the study as this plant can grow easily at any country which have tropical climates. In South America the most common diseases that cause by this cocoa plant are black pod, witches broom and frosty pod rot. To overcome this major issue, extensive study was carried out on the endophytic microbial community of the cocoa plant (Samuels et al. 2012a). From this study, it was concluded that several fungus strains such as *Trichoderma koningii*, *T. ovalisporum*, and *T. koningiopsis* can play its role as endophytes in the cocoa plant (Degenkolb et al. 2008).

According to the study done by Rubini et al. (2005), diversity study about endophytic fungi in the cocoa plant give rise for variety of fungal strains but low prevalence was given to the isolated strains. Posada et al. (2007) reported that there are few endophytic microbiota can be found in the coffee seedlings which contain some of the *Trichoderma* strains such as *Trichoderma harzianum* and *Trichoderma hamatum.*

Besides the cocoa plant, similar studies were also conducted on other plants. One of the good example for this scenario is the *Hevea* spp. which also known as rubber tree. According to Chaverri et al. (2011), a new species named *Trichoderma amazonicum* was isolated from this rubber tree. In Mexico, two other *Trichoderma* fungus functioned as endophyte in two different plants. Zhang et al. (2007) *Trichoderma* strain was identified from *Taxus mairei,* while Samuels et al. (2012b) identified *Trichoderma solani* from the tubers of *Solanum hintonii*. Six types of *Trichoderma* species were identified from the banana root where four of them were from the inside the root, while the rest two were isolated from the surface of the root. Among the species *Trichoderma asperllum* and *Trichoderma virens* showed the highest frequencies in the samples (Xia et al. 2011a, b).

According to Dang et al. (2010), *Trichoderma ovalisporum* was isolated from the traditional Chinese medicinal plant where the strain shows effective antibacterial activity against several pathogenic microorganisms such as *Staphylococcus aureus* and *Escherichia coli. Trichoderma* strains were also isolated from other important medicinal plants such as *Huperzia serrata and Salvia miltiorrhiza.* Strains also were detected from some of the carnivorous plants. Some studies were done on *Drosera rotundifolia* which is also known as common sundew during the season of autumn and spring by Quilliam and Jones (2010). *Trichoderma viride* was isolated from all the samples. Other carnivorous plant which is *Pinguicula vulgaris* was also studied for the potential endophytic fungi. The root and seed from *Dendrobium nobile* and

Dendrobium chrysanthum also studied for the presence of endophytic and mycorrhizal fungi (Ming et al. 2012). Numerous studies and efforts were taken to overcome the growth of the harmful pathogenic fungi, *Rhizoctonia solani.* This fungus has the ability to cause serious quality as well as quantity damages on the potato tuber. However, the endophytic fungi that isolated from the potato plants were tested and based on the obtained result, isolates of *Trichoderma atroviride* show the highest inhibition against *R. solani* due to the mycoparasitic phenomena (Chen et al. 2011).

8.3.3 Air and Settled Dust

Air plays pivotal role when it comes to the dispersal of spores and conidia of fungus. According to the study done by Madsen et al. (2007), *Trichoderma* species can be isolated from air of different environment which varies from outdoor to indoor air. Some good example of indoor air samples are those from heated wooden chips inside the buildings, from air filters that being used in hospitals, air ducts of the households, air conditioners, and achieve storage facilities. *Trichoderma* can be isolated from the outdoor air samples as well which normally from the rooftop of hospitals and houses. The air samples from agricultural area can be potential for this fungus isolation as well. According to Madsen et al. (2007) the dust blown from hay in England can be used for the isolation purpose. However, no species level identification was done for the samples that has been isolated from the air samples and settles dust. From the clinical wise, presence of conidia in the air plays a significant role for the occurrence of allergic diseases and can cause the infections like sinusitis or pneumonia. On the other hand, Hansen et al. (2010) concluded that *Trichoderma harzianum* which used as biocontrol agent can be detected in the air only during the treatment period.

8.4 Agricultural Habitats

Abiotic and biotic stress plays a significant role towards the population and diversity of any microbial communities in the agricultural ecosystems (Rana et al. 2019a, b; Yadav et al. 2018a). This includes the species of the plant and its growth stage, soil physical and properties of the chemical that being used, usage of pesticides, the geographical region and most importantly the total microbial competition (Druzhinina et al. 2011). *Trichoderma* spp can be isolated from any type of agricultural fields as it exhinited significant positive impacts on plant cultivation. Those impacts include its role as efficient biocontrol agent, induction of systemic resistance, increase the uptake of nutrient, promote the plant growth and degradation of any pesticides that can bring negative impact to the soil. The rhizosphere attracts this fungus by having the presence of different soil borne fungi which function as its prey and rich derived nutrient. The members of *Trichoderma* are preferred to be isolated from rhizosphere and non-rhizosphere soil rather than phyllosphere. Some of the species are ubiquitous where some are limited to certain geographical areas (Kour et al. 2019c).

As mentioned earlier, the isolation of this fungus from various agricultural crop fields was carried out to conduct studies on its biological control ability against phytopathogens, so only limited number of studies that deal with the population, abundance and diversity of the genus of *Trichoderma* from specific crop fields were carried out. *Trichoderma* spp can be highly found in cereal crop fields and is one of the dominating fungi and prevalent taxa among other fungal communities. When it comes to the cereal crop field, the weather, the type of soil and farming management bring big influence to the distribution of the *Trichoderma* spp. Six different species of the fungus were discovered by the ITS barcoding and restriction fragment length polymorphism (RFLP) from the rhizosphere, rhizoplane and the soil bulk of potato (Liang et al. 2004).

From the rhizosphere of coffee, *Trichoderma* isolates also have been discovered particularly from the soil of the coffee plants in the forest and semi-distributed forest area. The identification was done by ITS barcoding. Either its cultivated or uncultivated coffee regions, both of the different area show higher diversity of this isolates but the indices and the evenness for this isolates were the same at the both the habitats. Some chemical properties and the altitude of the samples concluded that the growth of the isolates was not preferred by the ecological perspective. It was also concluded that the host plant influence the genetic diversity of *Trichoderma* strains. Around 135 isolates of *Trichoderma* were collected from the rhizospheres of cocoa plant of different locations (Pavone and Domenico 2012).

From the rhizosphere of sugar beet, sixteen isolates were collected and the identification process was done based on the morphology, ITS and *tef1* analysis. When it comes to the oilseed rape rhizosphere, *Trichoderma* spp. were the most prevalent fungi and most importantly it showed high biodiversity and specificity in the bulk soil of oilseed rape. Another most important plant that acts as a rhizosphere for *Trichoderma* isolates is the oil palm. Among the isolates strains, *Trichoderma harzianum* and *Trichoderma virens* were the most prevalent species that had been recovered from the soils of oil palm in Malaysia. Population wise, it shows the high in both the cultivated and semi-cultivated oil palm ecosystems. However, the population increased when empty fruit bunches were added to the fields and the moisture content and the pH of the soil did not bring any effect towards the distribution of the strains. In conclusion the diversity and the abundance of the *Trichoderma* were higher in bulk soil than in rhizosphere soil (Naeimi et al. 2011).

8.5 *Trichoderma*: Promoting Healthy Plant Growth

In the recent time, *Trichoderma* spp. has been widely suggested to be used for efficient plant growth as a good plant growth promoting fungi (PGPF). These have capability of producing some of the secondary metabolites in particularly siderophores, phosphate-solubilizing enzymes and phytohormones for plant growth and soil health (Doni et al. 2013; Kour et al. 2019b; Yadav 2019). This plant growth promoting traits is considered one of their beneficial trait and this traits can be seen through different type of mechanisms such as antibiosis, degradation of toxins, mycoparasitism, inactivation of pathogenic enzymatic pathway and also but not least the enhanced nutrient uptake which leads to the overall development (Lorito et al. 2010; Kaur et al. 2020; Kumar et al. 2019; Singh et al. 2020). This fungus is known as hemibiotrophic fungus which is effective towards reducing the severity of the plant diseases by several mechanisms which have been mentioned above (Fontanelle et al. 2011). Most of the secondary metabolites produced by *Trichoderma* are strain dependent, can contain both volatile and nonvolatile antifungal substances and it is rhizosphere-competent strains which mainly involve in colonizing the surface root which have been shown for causing direct impact on the plants. It helps to increase the nutrient uptakes which eventually leads to better physical growth of the plant (Gal-Hemed et al. 2011).

According to Doni et al. (2014), the usage of *Trichoderma* as plant growth promoter shows significant positive impact towards the height of the plant, number of leaf and the length of the root. The possibilities for this to occur are mainly because of mechanisms which involve nutrient usage and the tolerance to abiotic and biotic stress. In addition to that, phytohormones produced by this fungus play a pivotal role for the enhanced growth of the plant. Some of the reports include phytohormones such as cytokinin like molecules and gibberellins related molecules can make biological enhancement on the crop fertility. On the other hand, Kashyap et al. (2017) also mentioned that the rice which inoculated with the *Trichoderma* shows higher photosynthetic rate. Other good impact shown by rice with the presence of *Trichoderma* is better uptake of the nutrients and increased resistance towards drought. On the other hand, maize plant, this fungus increased the plant's biomass production as well as developed better root hair. *Trichoderma* also helped to increase the seed germination process, increase the osmotic, salinity and chilling by initiate the physiological protection against any form of cellular damage (Mastouri et al. 2010).

The interaction in between *Trichoderma harzianum* strain and tomato-root system was also studied at the beginning stage of the root colonization. The presences of the fungus inoculation in the production medium cause the profuse adhesion of the hyphae to the roots and the colonization of the root epidermis and cortex. The green fluorescent protein (GFP) shows the hyphal growth and the formation of the papilla like hyphal tips which induced by the plant.

Trichoderma harzianum in particular is able to control and inhibit the growth of *B. cinerea* on grapes by colonize the blossom tissue and exclude the pathogen from the infected side. According to Kashyap et al. (2017), *Trichoderma* species used the competition for nutrient as their major mechanisms to inhibit the soil *microorganisms*. In addition to that, *Trichoderma* has the advantage of mobilizing and take up all the nutrients which makes much more efficient and more competitive than many other soil microbes. However, the biotic components of the soil have several impacts on the bio control activity of *Trichoderma* against other plant pathogens. According to Kashyap et al. (2017), when the *Gfp* contained mutant strain used, it shows higher level of microbial soil biomass that induce the shift from the hyphal growth to sporulate in *Trichoderma* (Table 8.1).

8.6 *Trichoderma*: Biological Control Agent

Fungi have been used for biocontrol purpose in much modern agricultural system. This is mainly because most of the fungi have the ability to reduce the negative impact of plant pathogen (Rana et al. 2020; Yadav et al. 2018b). *Trichoderma* sp. have gained much more interest than any other fungi due to their surviving ability under any form of unfavourable condition, high capacity for reproductive purpose,

Table 8.1 The effect of *Trichoderma* inoculum on the agronomical and physiological aspects of the crops

Trichoderma strain	Crop	Application mode	Effects	References
Trichoderma sp.	Chickpea	Agar plates were inoculated with a fungal mycelial disc of 5 mm diameter	Increased solubilization of inorganic phosphate	Rawat et al. (2011)
Trichoderma harzianum	Cucumber	5 × 106 conidia per g of soil or sprayed on roots at a concentration of 1 × 108 conidia/ml	Promoted seed germination, vegetative growth, and flowering	Chang et al. (1986)
Trichoderma harzianum	Chrysanthemum	5 × 106 conidia per g of soil or sprayed on roots at a concentration of 1 × 108 conidia/ml	Promoted seed germination, vegetative growth and flowering	Chang et al. (1986)
Trichoderma asperelloides T203	Arabidopsis	Root system was inoculated with a solution containing 1 × 10^5 spores/ml	Improved seed germination	Brotman et al. (2013)
Trichoderma hamatum	Arabidopsis	Trichoderma bran inoculum added to soil before sowing	Promoted root and shoot growth	Studholme et al. (2013)
Trichoderma atroviride	Lettuce	The substrate was supplied with prepared tablets containing 4.5 × 10^5 conidia	Enhanced shoot and root dry weight, and chlorophyll content	Colla et al. 2015

capability to resist against other plant pathogenic fungi and can able to produce secondary metabolites (Contreras-Comejo et al. 2016; Benítez et al. 2004). Besides that, this fungus has been also used in various biotechnological applications and used in agriculture field mainly to enhance the growth and the yield of the plants (Lorito et al. 2010; Hermosa et al. 2012).

Any form of penetration or interaction in plant roots normally will activate its immune system but in case of *Trichoderma* sp. it will remodel the immune system of the plant and help it to be recognized as non-pathogenic plant. The strains of the fungus can able to eliminate the synthesis of JA and ET which mainly involved in inducing systematic resistance (Hermosa et al. 2012). The systematic resistance induced by *T. asperellum* or *T. harzianum* T39 against *B. cinerea* in *A. thaliana* was determined as well. *Trichoderma* sp. plays an important role when it comes to suppressing the formation of any plant disease and plant pathogen in both the field condition and greenhouse condition. The fungus can produce wide range of elicitors which will interact with the receptors and recognition of *Trichoderma* and this will lead to the induction of resistance in the plant (Salas-Marina et al. 2011). In addition, different types of *Trichoderma* strains are able to produce little amount of secondary metabolites and these metabolites seem to induce the pathogenesis-related protein and eventually reduce the symptoms of any disease (Vinale et al. 2006). Through the mycoparasistm process, *Trichoderma* can parasitize the plant pathogen during different stages of development. This is done by producing several enzymes such as chitinase, proteases and glucanases which will help to flow the nutrient into the mycoparasite and finally degrade the host of the pathogen (Inch and Gilbert 2011). Table 8.2 shows the different *Trichoderma* strains against different bacterial pathogens.

Both the mono and dicotyledonous species show strong resistance towards attack of various pathogens when the plant is well pre-treated with *Trichoderma*. The plant colonization caused by this fungus inhibits the growth of different pathogens at the side of inoculation and also when the biocontrol fungus inoculated at different times or sites than that of the pathogen. In addition to that, the resistance of the plant shown by the colonization of some of the *Trichoderma* sp. is pretty much similar to that elicited by the rhizobacteria where it enhance the defense system of the plant but do not directly involve with the production of the pathogenesis-related proteins known as PR proteins. Recent studies have been done at the molecular level by using the high-density oligonucleotide microarray approach and it was found out that the gene induced by *Trichoderma* was associated with the biotic and abiotic stresses and also RNA, DNA and protein metabolism. The genes that codify for the extensin and also extensin look like proteins were mainly induced by the biological agents.

The plant starts to interact with *Trichoderma*, different types of metabolites which belong to different classes will start to be produced as elicitors or as resistance inducers (Woo and Lorito 2007). Proteins with enzymatic activity such as xylanase, avirulence are able to induce the plant defence mechanisms and induce the production of low molecular weight compound as a result of fungus–plant interaction. Some of these low molecular weight compounds which have the ability to

Table 8.2 Different *Trichoderma* strains against different bacterial pathogens

Trichoderma Strains	Plant	Pathogen	Mechanisms/ activity	References
Trichoderma harzianum	Tomato	*Clavibacter michiganensis subsp. michiganensis*	Lysosime and prevent activity	Utkhede and Koch (2004)
Trichoderma spp.	Rice	*Xanthomonas oryzae* pv. *Oryzae*	Biocontrol activity	Gokil-Prasad and Sinha (2012)
Trichoderma asperellum	Tomato	*Xanthomonas campestris pv. vesicatoria*	Biocontrol activity, antagonism dosage	Suárez-Estrella et al. (2014)
Trichoderma harzianum T23	Activity in vitro	*Clavibacter michiganensis* and *Erwinia Amylovora*	Production of viridiofungin A (VFA)	El-Hasan et al. (2009)
Trichoderma reesei	Arabidopsis and tomato	*Clavibacter michiganensis*	Production of extracellular enzyme, swollenin	Saloheimo et al. (2002)
Trichoderma asperelloides T203	Tomato	*Pseudomonas syringe* pv.	Increase level of WRKY 40 transcription factor	Brotman et al. (2012)
Trichoderma atroviride	Tomato	*Alternaria solani*	Secrete Sm1 and Ep11 protein	Salas-Marina et al. (2011)

degrade fungal cell wall have been purified and further characterized. These compounds have been mainly composed of oligosaccharides with or without amino acids moiety. In these interaction mechanisms *Trichoderma* activates the mycoparasitic gene expression cascade. Besides the activated antimicrobial effect, their action might stimulate the biological activity of the resident antagonistic microbial populations or the introduced *Trichoderma* strains and this will induce the effect of induced systematic resistance on the plant.

8.7 Biotechnological Applications

8.7.1 Production of Antibiotics and Other Secondary Metabolites

There are several compounds which are not involved for the normal growth and the reproduction of any organisms. These compounds are called as secondary metabolites. However, they play pivotal role when it comes to the development, signaling and the interaction between one organism and other (Rastegari et al. 2019; Yadav et al. 2019a; Saxena et al. 2016). The absence of these metabolites will result in the long term impairment on the survival of the organism rather than causing a direct and immediate death. According to Mukherjee et al. (2012), certain environmental factors will cause the organism to be dependent on the secondary metabolites such as in case of iron deficiency as siderophores act as replacement to support growth. Plants use these secondary metabolites as a defence system against herbivores and other inters species. According to Vipul et al. (2014), humans use it for medicine, flavourings and drug creation purposes. On the other hand, it also can be used as efficient weapon against other pathogenic bacteria, amoeba, fungi, insects and even large animals. Secondary metabolites can be used as agent for symbiosis purpose between plants and other organisms (Kour et al. 2019a).

Commonly known as filamentous fungi, *Trichoderma* spp., can be found nearly in most of the soils and other habitats. They have the capability of adapting to different ecological conditions and lifestyles. *Trichoderma* fungus has economical important mainly due to the fact that it can be used as a good biocontrol agent which help to inhibit the growth of phytopathogenic fungi. It can be functioned as biocontrol agent because of the presence of extracellular enzymes and secondary metabolites. Particularly the secondary metabolites produced by the fungus can be used in biotechnological and pharmaceutical purposes. This includes secondary metabolites such as peptaibols, non-ribosomal peptides, terpenes, steroid and many more. Although there have been 373 different molecules that have been identified so far, their specific activity is still cannot be determined.

According to Verma et al. (2017b), these fungal biocontrol agents have been mainly used against any soil related diseases. In order for them to perform as an efficient biocontrol agent they must show different mechanisms of action when it comes to their antagonistic interaction with any fungal pathogens. Some good examples are the mycoparasitism, antibiotic activity, basic competitions for nutrients, induction of systematic resistance and cell wall lytic enzyme activity. Trichodermin, gliovirin and harzianic acid are the best known metabolites that being produced by *Trichoderma* species (Singh 2010). Strains such as *T. viride*, *T. harzianum* and *T. atroviride* are capable of producing pyrone (6-pentyl-2H-pyran-2-one). These metabolites are the main reason behind the release of coconut aroma. Pyrone is also known for its antifungal activity against plant pathogenic fungi. The fungus also produces cytosporone which show strong in vitro antibiotic activity against some pathogenic bacteria and fungi.

Koninginis metabolites are also capable of inhibiting the growth of fungus such as *Gaeumannomyces graminis* var. It also can kill some of the soil-borne plant pathogens such as *Fusarium oxysporum*, *Pythium middletonii*, *Rhizoctonia solani* and many more (Vinale et al. 2006). These secondary metabolites in particular can prevent the spore germination by the fungal pathogens. According to Vinale et al. (2006), a secondary metabolite called T22 azaphilone from the liquid culture of *T. harzianum* was isolated. These metabolites inhibit several plant pathogens such as *R. solani* and *G. graminis* var. *Trichoderma hamatum* produces viridiol which reduces and at certain circumstances inhibits mycotoxin like aflatoxin which produced during the fungal interaction. So the usage of this secondary metabolites produced by *Trichoderma* strains can induce the resistance level of the host and also promote the yield of the crop which can be the most suitable replacement for the chemical usages. Due to the presence of the secondary metabolites, *Trichoderma* strains can also be used as biofertilizers where there are two strains, namely *Trichoderma harzianum* T22 and T39 used as active agents for various commercial biopesticides and biofertilizers.

Most of the fungus around the world has the ability to produce potential toxins in the form of antibiotics which can kill other pathogenic microbes regardless its concentration level. The differences in between these antibiotics somehow show different activities against both prokaryotes and eukaryotes. Paracelsin is the first antibiotic discovered that produced by the species *Trichoderma.* Most of the time it produces large compounds that have the antibiotic activity and this production is mainly dependent on the factors such as pH, temperature and quantity of microorganisms. A single *Trichoderma* species can produce different type of antibiotic compounds and likewise the given antibiotic has the potential of being produced by different strains. However, according to the study done by Sivasithamparam and Ghisalberti (2014), it revealed that different isolates of same species can produce different compounds as well.

Trichoderma harzianum often leads to the production of antibiotic where the methanolic extract from the dual culture of this fungus showed strong antimicrobial activity against *Staphylococcus aureus*. Moreover, general *Trichoderma* species that can be found in marine habitat also known to produce bioactive metabolites such as antimycobacterial. According to the study conducted by Wu et al. (2014), from the culture of marine *Trichoderma* strain tentatively named as MF106, the particular strain shows antimicrobial effects towards human pathogenic strains such as *Staphylococcus aureus.* The strains also known to be used for the protection purpose of grapevine wounds from the infected trunk pathogens. In a nutshell, *Trichoderma* spp. can be used on a wide spectrum for both in vitro and in vivo (Kotze et al. 2011).

8.7.2 Production of Hydrolytic Enzymes

Currently, most of important crops which have economic purposes largely are being destroyed by the pathogens fungi in particular which the most aggressive is soil-borne pathogen. There is some investigation being carried out in order to overcome this problem. One of the well-known steps is the usage of chemical pesticides to control the growth of these pathogenic microorganisms (Yadav et al. 2018a, b). However, using chemical pesticides can cause long term effect towards the human health as well as the environment. Thus, eco-friendly alternatives have been approached to replace the usage of the chemical pesticides.

*Trichoderm*a fungus can be an eco-friendly replacement for the chemical pesticides due to its ability of producing hydrolytic enzymes. The cell wall of the fungus can able to produce hydrolytic enzymes such as cellulase, chitinase and more. This enzyme plays a pivotal role when it comes to the degradation of biomass (Schuster and Schmoll 2010). The cell wall of *Trichoderma* is mainly made up of β-1,3-glucans and chitin and sometimes cellulose in some of the oomycetes. There are some demonstrations that have been done to give a clear picture on how some of the *Trichoderma* strains produce hydrolytic enzymes to inhibit the growth of pathogenic microorganisms such as in case of *Trichoderm harzianum*.

In most cases, the production of this enzymes mainly depends on factors such as availability of type of carbon source, presence of light, growth rate and secretion stress (Tisch and Schmoll 2013). Besides than the strain that have been mentioned above, *Trichoderma reesei* is another widely used strain which produces cellulose and the hemicellulose degrading enzyme. Both of these enzymes are highly used for industrial purposes. Häkkinen et al. (2012) mentioned that a complex system will be formed by the large number of carbohydrate active enzymes that being produced by the fungus which will be regulated by the series of environmental and physical factors. *Trichoderma hamatum* on the other hand possesses high antimicrobial activity mainly because it consists of specific β-glucanase and chitinase. Both of these enzymes play significant role when it comes to the cell wall degradation. According to Ahmed et al. (2009) three type of glucanases: exoglucanase (EXG), endoglucanase (EG), and β-glucosidase (BGL) have been isolated from *Trichoderma harzianum*. These enzymes also being used for malting, baking, alcohol production, paper and textile industries (Galante et al. 2014).

8.8 Conclusion and Future Prospect

For many years, based on its GRAS status according to FDA, Trichoderma has been applied in agriculture to promote cell growth and control of plant diseases without any potential risk to the environment. However, the non-agriculture applications of this microbe are still very limited even it consider as one of the safest microbes without any potential pathogenicity to human and animal. Based on the high capacity of *Trichoderma* for the production of hydrolysis enzymes, primary and secondary metabolites, it is strongly believed that this microbe has very high potential in biotechnology industries. In addition, this microbe has high capacity to grow on wide range of cheap substrates as a result of the high capacity to produce wide range of hydrolases enzymes to breakdown the complex carbon and nitrogen sources. The high capacity for enzyme excretion makes it also a very attractive biofactory for native and recombinant enzyme production. These all together giving us the strong confident of the high potential future application of *Trichoderma* not only in green biotechnology (agriculture) but also in white biotechnology (industry) for the production of their own native and recombinant products.

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