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Role of Cell-Wall Degrading Enzymes in Mycoparasitism

(*Prashantha S T and Jagdish Yadav)

Division of Plant Pathology, ICAR-IARI, New Delhi (110012), India

* prashanthst1144@gmail.com

A typical mycoparasitic interaction involves sensing of the host/prey fungus, attraction, attachment, coiling around and lysis brought about by hydrolytic enzymes, in many cases, in conjunction with secondary metabolites. Host cell-wall lysis is accomplished by the action of chitinases, glucanases (including cellulases), and proteinases and may also be combined with the action of antibiotics. Fungal chitinases are involved in different stages in fungal growth, including hyphal elongation, cell separation, branching, spore swelling, germination, sporangium formation and response to mechanical injuries. A glucanase that cleaves ß -1,3-glucan-type bonded glucans is a *ß -l,3-glucanase*, and a glucanase that cleaves bonds located within the chain are endo-glucanases. *Trichoderma* spp. secretes proteases in the presence of a host fungus and proteases have been isolated from several species of *Trichoderma*. Among the *Trichoderma sp., T. reesei,* is having high amount of cellulase activity, which highly utilized in industrial purpose (food, feed, textile and biofuels industries).

Mycoparasitism

Mycoparasitism, the direct attack of one fungus on another, is a very complex process that involves sequential events. A typical mycoparasitic interaction involves sensing of the host/prey fungus, attraction, attachment, coiling around and lysis brought about by hydrolytic enzymes, in many cases, in conjunction with secondary metabolites. The fungal cell wall is mainly composed of polysaccharides (80%) and proteins (3-20%), with lipids, pigments, and inorganic salts present in lower amounts. The main macromolecular components in the cell walls of higher fungi (Ascomycetes, Basidiomycetes, Deuteromycetes) are ß-glucan, chitin, and mannoproteins (glycoproteins). In the lower fungi (Myxomycetes, Phycomycetes), cellulose predominates over chitin. ß-glucan, chitin (chitosan in some fungi), and cellulose microfibrils form the scaffolding responsible for the strength and shape of the cell wall.

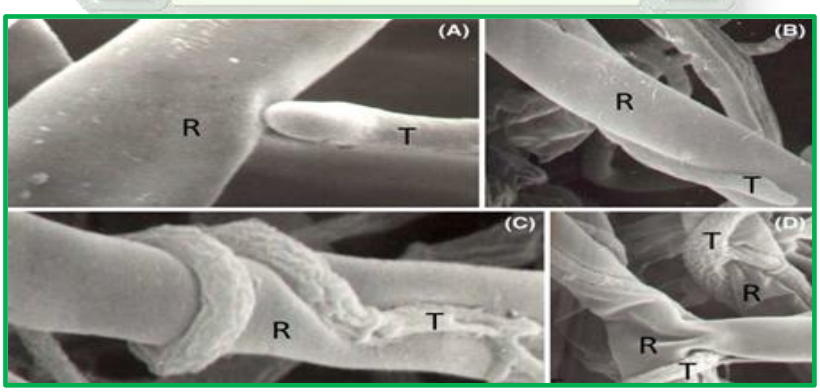

Fig.1 Mycoparasitism of *Trichoderma virens* (T) on *Rhizoctonia solani* (R)

Thus, host cell-wall lysis is accomplished by the action of chitinases, glucanases (including cellulases), and proteinases and may also be combined with the action of antibiotics. Penetration of the cell wall has often been observed, and parasitising hyphae visualised growing inside the host hyphae.

Cell-wall-degrading enzymes: chitinases

Chitin polymers are an essential structural component in the fungi kingdom, as well as in some members of the animal kingdom. In fact, chitin is one of the most abundant polysaccharides found in nature, second to cellulose. The degradation of chitin is catalyzed by chitinolytic enzymes, called chitinases. Fungal chitinases are involved in different stages in fungal growth, including hyphal elongation, cell separation, branching, spore swelling, germination, sporangium formation and response to mechanical injuries. Haran *et al.* (1996) divided these enzymes into three classes. The first class contains the enzymes that work in exo-type to produce GlcNAc monomers. This class of enzyme, known as 1,4-B- N-acetylglucosaminidases [also known as hexosaminidase], is responsible for cleaving the terminal chain of chitin to release N-acetylglucosamine. The second class contains chitinases that cleave inside the chitin chain in internal sites and these enzymes are called endochitinases. The last class is known as exochitinases or chitobiosidases and responsible for cleaving the chitin to produce dimers of GlcNAc units but no monomers or oligomers.

a. 1,4-β **N –acetylglucosaminidases**

Many GlcNAcases and their genes—exc1 (=nag1), exc2, tvnag1, and tvnag2 from *T. harzianum* T25-1, *T. atroviride* P1 and *T. virens* Tv29-8 —have been described. The 73-kDa Nag1 represents the main GlcNAcase in *T. atroviride*. Nag1-disruption strain lacks chitinase activity, and the endochitinase chit42 mRNAis absent. This indicates that nag1 is essential for triggering chitinase gene expression. The pathogen cell wall and chitin induce *nag1*, but it is only triggered when there is contact with the pathogen.

b. Endochitinases

Several endochitinases have been isolated from *T. harzianum* such as the endochitinases of molecular weight 37 and 33 kDa and 31 kDa. Also, the 42 kDa endochitinase (ech42) has been isolated from various Trichoderma species included *T. harzianum*. Ech42 is involved in the mycoparasitic action of *Trichoderma* on three levels, including cell wall hydrolysis, spore germination inhibition and the inhibition of the elongation of germ tubes of several filamentous fungi. The expression of this enzyme is highly induced during the interaction between the mycoparasite and its host, suggesting that the ech42 is a key enzyme during the parasitic interaction.

Cell-wall-degrading enzymes: glucanases

The second group of enzymes that is important for the mycoparasitic activities of *Trichoderma* spp. is the glucanases. The fungal cell wall is composed mainly, along with chitin, of B- 1, 3-glucan (laminarin) and 1,4-fi-D-glucan (cellulose). Glucan is a major component in fungal cell walls and its primary role is to provide structure, rigidity and protection. The synthesis of glucan-degrading enzymes is a shared characteristic between many organisms, although fungi are the principal producers. Glucanases are classified according to the type and location of glycosidic linkages that they cleave. A glucanase that cleaves ß -1,3-glucan-type bonded glucans is a *ß -l,3-glucanase,* and a glucanase that cleaves bonds located within the chain are endo-glucanases. Thus, endo- ß -1,3-glucanase cleaves covalent bonds in the triple helical structure of D-glucose molecules in a ß-configuration, or ß-1,3-glucans. In fungi, ß -1,3 glucan degrading enzymes are prevalent, due to the vast availability of their substrates in fungal cell walls. ß-1 ,3-glucanases that hydrolyse laminarin have been subdivided into two classes, endo and exo- ß glucanases based on the activity

against the substrate. Endo- ß -glucanases hydrolyse laminarin into oligo saccharides whereas exo- ß -glucanases produces monosaccharides, since they cleave the terminal units. Both the endo- ß -glucanases and the exo- ß glucanases have multiple roles in fungi and are involved in the differentiation and metabolism of ß-glucans. The formation of ß -1,3-glucanases is inhibited by glucose, a feature that glucanases share with chitinases. A second class of glucanase enzymes that is also produced by *Trichoderma* is the ß-1,6-glucanases. These enzymes work on the minor structural polymers that are present in the fungal cell wall. Various ß-glucanase activity levels were induced against cell-wall preparations from several pathogens. Moreover, these enzymes play a role in the *Trichoderma* antagonism of *Pythium*. *Pythium*, an oomycete contains a cell wall that is a mixture from B-1,3-glucans and 1,6 glucans, in addition to cellulose, but not chitin. Glucanases enzymes are important for the lysis of the host fungi cell walls during the mycoparasitic interaction.

Cell-wall-degrading enzymes: Proteases

Fungal cell wall structure is sustained by various proteins and glycoproteins. Consequently, proteases are considered to be factors in fungal cell wall degradation. Therefore, the presence of protease enzymes in concert with chitinases and glucanases has been shown to increase subjectivity of host cell walls lysis. *Trichoderma* spp. secretes proteases in the presence of a host fungus and proteases have been isolated from several species of *Trichoderma*. The penetration into the host fungus is facilitated by the effect of protease that breaks down the peptide bonds in the host cell walls. Prb 1 is a 31 kDa alkaline protease, one of the hydrolytic enzymes produced by *T. harzianum*. The prb 1 gene was found to be active when the fungus is grown on media containing *Rhizoctonia solani* cell walls. Over expression of the prbl gene in *T. harzianum* was also used to improve the biological control of soil-borne plant pathogens. Another serine protease (tvspl) has been identified from *T. virens*. This Trichoderma species is used as a biocontrol agent, since the over expression of the extracellular serine protease gene tvspl can protect against *R. solani*. In addition, it is suggested that the inhibition of the enzymatic activity of the phytopathogenic fungus *Botrydis cinerea* by *T. harzianum* is due to the proteolytic enzymes that are secreted and degrade the *B. cinerea's* hydrolytic enzymes.

Cell-wall-degrading enzymes: cellulases

Cellulases enzymes are having three different classes. The first clase is ß-1,4-D-glucan cellobiohydrolases which is exocellulases, it cleaves cellobiose units from the ends of cellulose and its oligomers. The second class is Endo-ß-l,4-glucanases, which cleave ß linkages internally at random. The third class is ß-1,4-glucosidases, cleaving cellobiose units in to glucose units. Among the *Trichoderma sp.*, *T. reesei*, is having high amount of cellulase activity, which highly utilized in industrial purpose (food, feed, textile and biofuels industries).

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