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Deciphering the protein translation inhibition and coping mechanism of trichothecene toxin in resistant fungi

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ABSTRACT

In modern times for combating the deleterious soil microbes for improved sustainable agricultural practices, there is a need to have a proper understanding of the plant-microbe interactions present in the rhizospheric microbiome of the plant roots. In the present study, the interactions of trichodermin with petidyltransferase centre of ribosomal complex was studied by molecular dynamics and *in silico* interaction methods to demonstrate its mechanism of action and to decipher the possible reason how it may inhibit protein synthesis at the ribosomal complex. Further we have illustrated how trichodermin resistance protein (60S ribosomal protein L3) helps to overcome the deleterious effects of trichothecene compounds like trichodermin. Normal mode analysis of trichodermin resistance protein and 25S rRNA that constitutes the petidyltransferase centre showed that the W-finger region of the protein moved towards 25S rRNA. Further analysis of molecular dynamics simulation time frames showed that several intermediate states of large motions of the protein molecules towards the 25S rRNA which finally blocks the binding pocket of the trichodermin. It indicated that this protein not only changes the local environment and conformation of the petidyltransferase centre but also restrain trichodermin from binding to the 25S rRNA at the petidyltransferase centre.

1. Introduction

In the present agricultural practices, for efficiently regulating the effect of the microbes on the crops, there is a need to better understand the plant-microbe interactions at the molecular levels. The microbes inhabiting the rhizosphere of the crop plants are beneficial as well as harmful to the plants (Kumari et al., 2016). For instance, *Fusarium* spp. are one of the most studied plant pathogenic soil fungi that cause head blight disease in the crop plants, wherever *Trichodrema* spp. act as biofertilizers and bio-control agents by increasing nitrogen uptake, synthesis of phytohormones, solubilization of phosphate, iron chelation, secretion of secondary metabolites and inhibiting the microbial pathogens (Ahmed and Upadhayay, 2009; Bowen and Rovira, 1999; Gao et al., 2002). Trichothecene molecules (secondary metabolites) including Harzianum A, HT2-toxin, Neosolaniol, T2-toxin, Trichodermin, Monoacetoxyscripenol, Nivalenol, Deoxynivalenol, crotocin *etc* are secreted by rhizospheric soil fungi belonging to the family Hypocreacae (Alexander et al., 2011; Audenaert et al., 2013; Cardoza et al., 2011; Ismaiel and Papenbrock, 2015; Kimura et al., 2007; Malmierca et al., 2013; Strub et al., 2010; Tijerino et al., 2011). Recent studies have indicated that the potential protein involved in the transport of these molecules is encoded by *tri12* gene present in *Trichoderma* spp. This protein belongs to major facilitator superfamily proteins that show structural and functional resemblance to the drug efflux pumps (Chaudhary et al., 2016; Sandhu and Akhter, 2016). Some of these molecules are also reported to be the inhibitors of protein translation in eukaryotic cells (de Loubresse et al., 2014). T-2 toxin produced by the fungi namely, *Fusarium graminearum*, *F. avenaceum*, *F. culmorum etc*. has been shown to bind to the peptidyltansferase centre (PTC) at the 60S tRNA A-site of *Saccharomyces cerevisiae* (de Loubresse et al., 2014). It was also reported that the core structural scaffold of trichothecene inhibitors mediates the major interactions with 25S rRNA residues in the binding pocket of the PTC of ribosomal complex of yeast (de Loubresse et al., 2014). There are reports indicating phytotoxicity of trichodermin on the plants as well as inhibition of some of the pathogenic fungi (Bowen and Rovira, 1999; Cardoza et al., 2011; Jain et al., 2013; Kumari et al., 2015; Malmierca et al., 2013; Shentu et al., 2014; Tijerino et al., 2011). Since both T2-toxin

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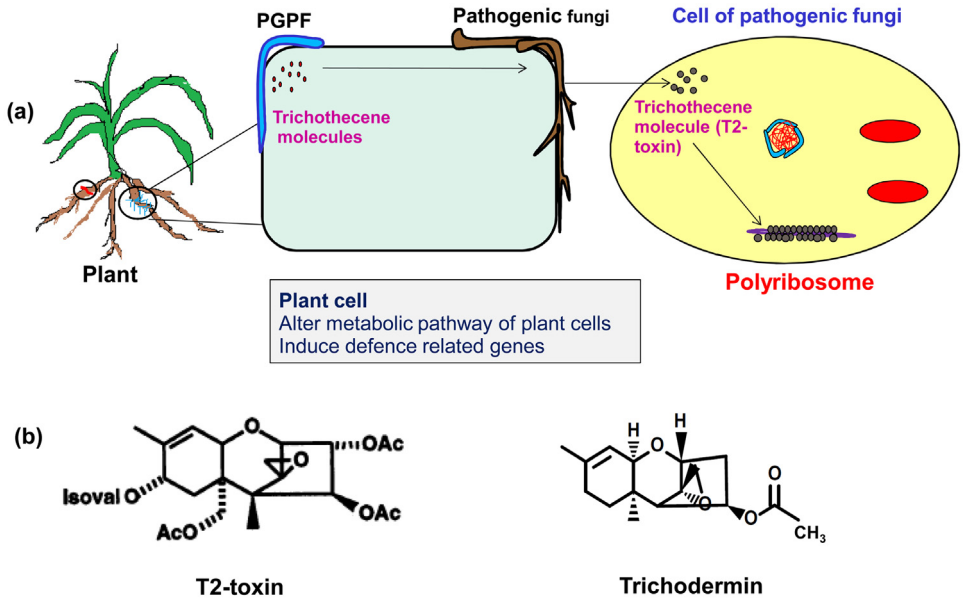

Fig. 1. Effects of trichothecene molecules on the pathogenic fungi: (a) Trichodermin, a secondary metabolite secreted by *Trichoderma* spp. which is present in the rhizosphere of the plant. It was purposed that these molecules act as pathogen associated molecular patterns which are reported to trigger defense related genes of the plants (Hermosa et al., 2013). Trichodermin has antifungal activity to inhibit growth of the pathogenic fungi (Malmierca et al., 2016; Tijerino et al., 2011). Trichodermin belongs to the same family of molecules as T-2 toxin *i.e.* trichothecene. It was reported that T2-toxin binds to 25S rRNA at the PTC of the yeast and inhibit protein translation (de Loubresse et al., 2014). Since the basic core chemical structure of T-2 toxin and trichodermin is same, we assume that it will also inhibit protein synthesis by binding to 25S rRNA at the PTC. (b) Chemical structure of trichothecene molecules *i.e.* T2-toxin and trichodermin showed common core. Therefore, their mechanism of action is purposed to be similar.

and trichodermin belong to same family of compounds and share same core chemical structure and hence, possibly trichodermin will also follow same molecular mechanism against the growth of the pathogenic fungi as reported earlier (de Loubresse et al., 2014) (Fig. 1). However, still there is no known mechanism that can explain in detail the events occurring at the molecular levels involved in the interaction of trichodermin with the pathogenic fungi resulting in their decreased growth. It was found that 60S ribosomal protein L3/trichodermin resistance protein (TR) present in the ribosomal complex of the yeast played an important role in amino acid-tRNA binding, peptidyltransferase activity, drug resistance, translational frame maintenance, virus replication and acts as a binding site for a ribosome inhibitory protein (Petrov et al., 2004). There is a universally conserved central extension of TR protein, also called as tryptophan- or W-finger, that projects to the A site of the PTC, where the tryptophan located at its tip closely approaches the PTC active site (Petrov et al., 2004). It was shown that it favours aa-tRNA binding and induced resistance in the ribosomal complex towards protein translation inhibitors (Petrov et al., 2004). However, these compounds have been well documented to inhibit protein translation in the fungi (Pusztahelyi et al., 2015). In the present study, we have used the ribosomal complex of *Saccharomyces cerevisiae* as a model to investigate the effects of trichodermin molecule on the A-site of the ribosomal complex. We have been able to demonstrate how TR protein protects the site of amino acid transfer from trichodermin in the ribosomal complex. To study this, we have employed molecular dynamics (MD) on the ribosomal complex of the yeast. Further it was shown by normal mode analysis (NMA) that the W-finger of trichodermin resistance protein moved towards the PTC of ribosomal complex and may aid in overcoming the effect of trichodermin in the fungi.

2. Experimental procedures

Molecular dynamics study on the ribosomal complex was carried out on the crystal structure of yeast (PDB ID: 4U6F) obtained from protein data bank (de Loubresse et al., 2014).

2.1. Docking of trichodermin and molecular dynamics of RNA-trichodermin complex

Trichodermin (ligand) was docked into the PTC (receptor) of the ribosomal complex of yeast using AutoDock Vina-2.0 software which is Monte Carlo based. The .pdb file of receptor and ligand was converted to .pdbqt file using AutoDock utility of MGLTools-1.5.6 (Morris et al., 2009). These .pdbqt files of receptor and ligand were added to conf.txt file which is used as input for AutoDock Vina. A grid was generated around the biding site of the dimensions 30, 30 and 30 in x, y and z directions respectively. AutoDock Vina used semiempirical free energy force field to evaluate the ligand binding conformation. Lamarckian Genetic Algorithm (LGA) was used to generate the binding poses (Trott and Olson, 2010). The best docked conformation was used as the starting structure for (MD) simulations. 25S rRNA and trichodermin complex was simulated with GROMACS 4.6 software using charmm27.ff force field (Berendsen et al., 1995; Vanommeslaeghe and MacKerell, 2015; Sandhu and Akhter, 2015). The toplogy file of ligand was generated using SwissPARAM server. The SPC water model was used to solvate the system, in a cubic box which was further subjected to steepest descent energy minimization for 50000 steps. The system was neutralized by addition of Na counter- ions. The system was subjected to the equilibration and production phases. The equilibration step consisted of the position-restraining of the ligand during the dynamics simulation (NVT and NPT) at 300 K for 100 ps. Finally, the whole system was simulated for the MD production run at 300 K temperature and 1 bar pressure for 30000 ps. For trajectory analysis, the atom coordinates were recorded at every 10 ps during the MD simulation.

2.2. Normal mode analysis of RNA-TR protein complex

To analyse the global motions of the flexible regions of the molecules, NMA with NOMAD-Ref server (which is based on the elastic network model and default settings was used for the parameters (Lindahl et al., 2006). Concisely, all the atoms of protein and