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The Insight of Mycovirus from *Trichoderma* spp.

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Abstract

Trichoderma spp. are used extensively in agriculture as a biological control agent to prevent soil-borne plant diseases. In recent years, mycoviruses from fungi have attracted increasing attention due to their effects on their hosts, but *Trichoderma* mycoviruses is in the beginning stage as the subject of extensive study. At present, eight researches were on the mycoviruses from *Trichoderma* spp. techniques of genome sequencing, elimination of dsRNA, detection of dsRNA, transmission of mycovirus were elaborated. With the deep research on the mycovirus, more and more effective methods for these basic researches should be applied. The topics about antagonism and biocontrol function of mycovirus will better push the deep exploration on the interaction among Trichoderma-mycovirus-plant (or pathogen), which also will have the driving role on seeking and screening more resources of *Trichoderm* spp. possessing biocontrol capabilities with mycoviruses.

Keywords: *Trichoderma* spp., Mycovirus, dsRNA, Application

Introduction

Viruses are popular small organism, which are distributing in human, animals, plants and insects to microorganisms, including bacteria, archaea and fungi and induced the obvious disease or symbiosis with the hosts without any symptoms [1-4]. Mycovirus is a group of viruses that can infect and replicate in filamentous fungi, yeasts and oomycetes and is widespread [3,4] which was first described by Hollings et al. (1962), who found three kinds of spherical or short rod-shaped viruses related to diseases in cultivated mushrooms [5]. Lampson et al. (1967) discovered a mycovirus in *Penicillium funiculosum* (Eurotiales, Ascomycota) and showed that it can induce an interferon-mediated response in the host [1,2,5-7]. In the same year, Ellis et al. (1967) observed the presence of virus particles in the culture fluid of *P. stoloniferum* by electron microscopy [8]. Based on previous studies, Banks et al. (1968) found that both *P. stoloniferum* virus (PsV) and *P. funiculosum* virus (PfV) had a dsRNA genome [9]. Since then, various types of mycoviruses have been reported. Until now, the classification of mycoviruses was based on the mode of replication and the type of the genome, which was divided all currently known mycoviruses into 16 families and an unclassified group by the International Committee on Taxonomy of Viruses [10]. The 16 families are consisted with seven dsRNA virus families, five positive-sense ssRNA virus families, two reverse-transcribing virus families (+ssRNA), one negative-sense ssRNA virus family, and one positive-sense ssDNA virus family [10]. The taxonomic status of roughly 20% of fungal viruses still need to be determined in the future [11,12].

The transmission and function of mycovirus: The transmission of mycoviruses have two ways, vertical and horizontal transmission. Vertical transmission is through spores of the fungi, including both sexual and asexual spores. In case of the mycelial asexual spores, the virus can be transmitted through the cytoplasm. This mode of transmission is relatively easy and especially common for dsRNA virus [13]. Horizontal transmission is accomplished by the fusion between hyphae, but this mode of transmission is limited by the incompatibility between the vegetative forms [14]. In some cases, few mycoviruses from fungi and fungi-like protozoans could not be virulent for the hosts with effecting the host fitness, including improving mycelia growth or reducing growth, abnormal pigmentation or deficient sporulation [4,6], and most mycovirus infections are asymptomatic [15]. But some mycoviruses had virulence, there were two main affections to plant pathogenic fungi: first, they can cause the host to become a low-virulence strain; second, the metabolites induced by the mycovirus can increase the pathogenicity of the host [16-18]. The most successful mycovirus biocontrol agent to date has been

Cryphonectria parasitica hypovirus 1 (CHV1), which was the first low virulence mycovirus employed to both prevent and treat disease [19]. Although some low virulence mycoviruses have been found in phytopathogenic fungi, most presently are still in the research stage as potential biocontrol agents.

The researches of mycovirus from Trichoderma: Despite Trichoderma spp. is researched popularly in the world for the function of biocontrol agent, and for producing important industrial enzymes [20-23], mycoviruses from Trichoderma spp. have been poorly studied and characterized. So far, there are eight descriptions of researches about *Trichoderma* mycoviruses [3,4,24-29].

The genome sequences of Trichoderma mycovirus: The first report for Trichoderma mycovirus was from the research of Jom-in in 2009 [24], however the signs of mycoviruses existing in *Trichoderma* spp. were only explored by checking the dsRNA by extraction methods, the genome sequences was not get anymore. Until 2016, Yun et al. still used the dsRNA extraction method to get 32 strains with dsRNA- mycoviruses from 315 strains of *Trichoderma* spp. from *Lentinula edodes* in Korea [25]. According to the diversification of number and size of dsRNAs among isolates, the band patterns of the dsRNA were categorized into 15 groups. The genome sequence was also not get yet.

The first whole genome sequences of the Trichoderma mycovirus was obtained from Lee's research in 2017 [26]. The complete genome is consisted by 8566bp, which contains two open reading frames (ORF), encoding structural proteins and RNA dependent RNA polymerases (RdRP), respectively. Phylogenetic analysis classified it belong to the family *Fusagraviridae* and named Trichoderma atroviride mycovirus 1 (TaMV1) [26]. In this research, the detection method for dsRNA was the electrophoresis, and then subjected to reverse transcription and cDNA library synthesis by using random hexanucleotide primers and reverse transcriptase, then RACE Analysis was used for 5'- and 3'-terminal sequences. This method was also used in the later following researches for sequences.

From then on, the five genome sequences of Trichoderma mycovirus were come out one after another. In 2018, Chun et al. obtained two genome sequences from mycovirus of Trichoderma, Trichoderma atroviride partitivirus 1 (TaPV1) [23] and Trichoderma harzianum partitivirus 1(ThPV1) [28]. TaPV1 was from *T. atroviride* and had two segments. The bigger one (dsRNA1) is consisted with 2023bp with one open reading frame (ORF) encoding RdRP. The smaller one (dsRNA2) has a total length of 2012bp with a single ORF encoding CP. Phylogenetic analysis indicated that the virus was a new member of *Alphapartitivirus* in the Partitividae family [27]. Moreover, the electron micrographs of purified viral particles of TaPV1 was shown as an isometric structure approximately of 30 nm in diameter. It was the first successful extraction of mycovirus particles from Trichoderma. ThPV1 was from *T. harzianum*, which is consisted of two dsRNA with similar sequence size. The larger dsRNA1 is 2289 bp with a single open reading frame encoding RdRP. The smaller dsRNA2 with 2245 bp contains an ORF encoding capsid protein (CP). Phylogenetic analysis indicated the virus was a new type of fungal virus which was not specifically classified into species in the genus *Betartitivirus*, family Partitividae, named Trichoderma harzianum partitivirus 1(ThPV1) [28]. All of these two mycoviruses possessed two segment, belonging to family Partitividae.

At the same time, a new fungal virus isolated from *T. asperellum* was reported in the laboratory of Guizhou Medical University, China, which was named Trichoderma asperellum dsRNA virus 1 (TaRV1) with two ORF on its genomic plus strand. ORF1 is a hypothetical protein, ORF2 encodes an RdRP. Based on RdRP sequence, phylogenetic analysis TaRV1 belongs to unclassified virus [29], which was the first report about Trichoderma mycovirus from China.

In 2019, Liu et al isolated two unclassified dsRNA mycovirus isolates harzianum mycovirus 1 (ThMBV1) [3] and harzianum mycovirus 1 (ThMV1) [4] from the 155 *Trichoderma* spp. strains, which were collected in the soil from Xinjiang and Inner Mongolia, China in 2019 [3,4]. The metagenetics as new method was used for checking the sign of mycovirus in strains, then electrophoresis, RT-PCR, 5' RACE and 3' RACE were used for whole genome sequence [3,4]. ThMBV1 was a new type of virus with bipartite segments mycovirus. one was 2088 bp encoding the RNA-dependent RNA polymerase (RdRP), and another segment was 1634 bp encoding a hypothetical protein. phylogenetic analysis suggested it was identified as unclassified mycovirus, named Trichoderma harzianum bipartite mycovirus 1. The phylogenetic analysis indicated it belonged to an unclassified family of dsRNA mycoviruses [3]. ThMV1 had two ORFs on the negative strand, ORF-A (residues 1857-109) encoded RdRP, ORF-B encoded a putative protein. On the positive strand, there was an ORF C (residues 1076-1370), presumed to be a hypothetical protein containing 94 amino acids, with the poly(A) structure on the 3' terminal. This was the first report about the RdRP and CP encoding on the negative strand of mycovirus genome sequence.

The Methods of Eliminating dsRNA

For the elimination of dsRNA is very important step for exploring the function of the Trichoderma strains with and without dsRNA. The basic method should be single-spore isolation followed by hyphal tipping, the auxiliary measures were always variated. Some used heat therapy, some used cycloheximide or ribavirin, and some also have been helped by the protoplasting/regeneration. In the research of Jom-in [24], the method of elimination of dsRNA was heat therapy, though not successful. The details were alternately heating at 37°C for 3 hours and room temperature (28-30°C) interval for 24 hours, and then like this for 10 times, but he did not use single-spore isolation. In the research of Yun et al [25], the elimination of dsRNA from strains is different with Jom-in, the auxiliary measures were depended on the cycloheximide (5 and 10μg/ml) or ribavirin (10 or 20mg/ml), which were used to eliminate the dsRNA incubated at 25°C, transferred agar plugs from the margin of each colony to the fresh V8 agar plates (100×15mm) [30] with the same concentration of

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either cycloheximide or ribavirin for 3-4 times, and then grown for 3 or 4 days in each time, and then hyphal tipped and grown in 50 ml of potato dextrose broth (PDB) (Difco Laboratories, Detroit) at 25°C for 2 weeks. The mycelium was used for further analysis of dsRNA presence [30]. Until 2018, the method of single-spore isolation followed by hyphal tipping from protoplast was successfully single used to eliminate dsRNA by Chun et al [27,28]. In our research, the ribavirin, protoplasting/regeneration and single-spore isolation followed by hyphal tipping were together used to successfully eliminate ThMV1 from Trichoderma strain 525 [4], but for ThMBV1, this method was not successful [3]. Moreover, RT-PCR and northern blotting should be a good method for detecting dsRNA, in the researches of Chun, Jem-in and Liu, it was good use of checking existing of dsRNA [20] and elimination of dsRNA [3,4,27,28].

Transmission of Trichoderma Mycovirus

The researches on the Transmission of Trichoderma mycovirus were limited. Normally, most vertical transmission of dsRNA into asexual spores of ascomycetes occurs at a markedly higher rate, for instance, the TaMV1 has a very high transmission rate 33/38 [26], but ThPV1 had low transmission rate into conidia with exceptional considering, the low transmission rate of ThPV1 into conidia is attributable to the intrinsic characteristics of the virus–fungus (ThPV1–*T. harzianum*) interaction [27]. We also found the transmission between same species also had the difficulties for ThMV1 (not published).

The Antagonism and Biocontrol Researches of Trichoderma Mycovirus

The antagonism and biocontrol researches were involved *in-vitro* and *in-vivo* researches, some differences were found between with and without strains, and some not. Jom-in comparing with the free isolates without the dsRNA, the function of the isolates of TM10 and TM20 with dsRNA reduced the host growth rate, sporulation and biological control efficacy [24]. But in the research of TaPV1[27], no apparent difference in colony morphology was observed between TaPV1-containing and the three virus-cured strains, moreover, β-1,3-glucanase and chitinase, as the two representative antifungal enzymes, no obvious alterations of enzymatic activities were observed between the infected and virus-cured isogenic strains [2018a], Moreover, the enzymatic activities of β-1,3-glucanase and chitinase were no changes in virus-cured strains, which was the first report of an *Alphapartitivirus* in *T. atroviride* [27]. In our research, antagonism characteristics of ThMV1 was explored though *in vitro* and *in vivo*. *In vitro* experiment, There were no obvious differences, when we tested the antagonism of T525 with ThMV1and T525-F without ThMV1 to three pathonogenic fungi (*F. oxysporum* f.sp.*cucumebrium* Owen, *B. cinerea* and *F. oxysporum* f. sp. *vasinfectum*). but *in vivo,* the removal of ThMV1 from host strain 525 reduced host biomass production and improved the biocontrol capability of the host on *Fusarium oxysporum* f. sp. *Cucumerinum*. Moreover, the presence of ThMV1 functioned to improve the growth of the cucumber [4]. It was the first research on detecting the changes of biocontrol *in vivo* of the Trichoderma trigged by mycovirus currency.

Prospect of the Study on Trichoderma Mycoviruses

At present, the researches of Trichoderma mycoviruses are limited, the more molecular technique for extraction of dsRNA, the genome sequences, eliminating methods, Transmission methods, the antagonism and biocontrol aceessment of Trichoderma mycovirus will be improved and abundant. In the next, the research goals of Trichoderma mycoviruses should be focus on these aspects:

a. For the resources of Trichoderma mycovirus should be rich in the nature, there should be more dsRNA mycovirus need to be explored in the future;

b. It is the total tendency to find more strains of *Trichoderma* spp. with mycoviruses with biocontrol function, which was hoped to the find more mycoviruses mediating the capabilities of Trichoderma to control plant disease, promoting plant growth and going through the environment stress;

c. The interval mechanism among Trichoderma-mycoviruses-plant-pathogen need to be discovered, for the complicated factors, the true interaction between or among the factors will be discovered with the progress of molecular biological informatics, transcriptome, proteomics and metabonomics;

d. Until now the resources of Trichoderma mycoviruses are limited, with the richness of them, the origin and phylogenetic research of the Trichoderma mycoviruses need to be more focuses for the growing enormous taxonomy in the future.

All in all, according to an applied agronomical perspective, discovering more mycoviruses infecting *Trichoderma* spp. populations and characterizing the nature of their host-parasite interactions would be of special interest for identifying new agents with potential biotechnological interest and also to better understand the ecology and temporal dynamics of fungal communities in natural and agronomic ecosystems.

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