



Research Article

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# The Exploration of Mycoviruses From Trichoderma Spp.



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## Abstract

Fungal mycoviruses have been research hot field because of their effects on the hosts, however the research of mycoviruses from biocontrol fungi of *Trichoderma* spp. is still in the initial stage. In this issue, a total of 144 strains of *T. spp.* collected from the soil of Xinjiang, Inner Mongolia and Tibet and were analyzed for the presence of double-stranded RNA (dsRNA). Three *Trichoderma* strains carrying dsRNA mycovirus have been recovered, which laid a good basis for the next research of complete genome sequencing, elimination, and its effect on the biocontrol function of *Trichoderma*.

**Keywords:** *Trichoderma* spp., dsRNA mycoviruses, exploration

## Introduction

Mycoviruses are a kind of widespread virus, which can infect filamentous fungi and yeasts [1-3]. Most viruses can infect the host without obvious infection symptoms [1-2]. Double-stranded RNA (dsRNA) genomes always the main nucleotide style of mycoviruses, which has been a marker of the existence of viruses [4]. The International Committee on Taxonomy of Viruses has divided all known branching viruses into 16 families and one unclassified group according to the mode of replication and genomics types of viruses [5]. At present, nine mycoviruses infecting *Trichoderma* spp. have been described [6-14]. 2009, Jom-in and Akarapisan provided the first description of two mycoviruses with sizes of 0.7kb and 1.1KB isolated from *T. spp.* [6]. Later, Yun et al. proposed that *Lentinus edodes* mycoviruses were widespread in Korea, and 32 different dsRNA-containing viruses were isolated from 315 strains of *T. spp.* [7]. Then, Lee et al. isolated an unclassified endovirus from *Trichoderma atroviride*, named *Trichoderma atroviride* mycovirus 1 (TaMV1) [8]. In 2018, Chun et al. obtained the complete genome sequences of two *Trichoderma* endoviruses, namely *Trichoderma atroviride* partitivirus 1 (TaPV1) and *Trichoderma harzianum* partitivirus 1 (ThPV1) [9,10]. Meanwhile, a new mycovirus named *Trichoderma asperellum* dsRNA virus 1 (TaRV1) was reported in the laboratory of Guizhou Medical University in 2019 [11]. In 2019, Liu et al. isolated two unclassified dsRNA endoviruses, *Trichoderma harzianum* mycovirus 1

(ThMBV1) and *Trichoderma harzianum* mycovirus 1 (ThMV1), from 155 *Trichoderma* strains which were collected from the soils of Xinjiang and Inner Mongolia, China [12,13]. In 2019, You et al. from Huazhong Agricultural University isolated a new mycovirus from *Trichoderma harzianum*, named *Trichoderma harzianum* hypovirus 1 (ThHV1), which may be a member of Betahypovirus [14]. In this paper, we chose 120 strains of *T. spp.* isolated from Xinjiang, Inner Mongolia, and Heilongjiang of China, to screen mycoviruses using molecular biology methods, and three mycoviruses strains were recovered.

## Materials and methods

### Fungal strains

One hundred and twenty *T. spp.* strains were isolated from the forests and grasslands of Xinjiang, Inner Mongolia and Heilongjiang of China, in 2017–2018. The species of all of these strains were identified by the lab experiment, the information about these strains were in the supplemental table S1. All fungal strains were cultured on potato dextrose agar (PDA) plates for 7 days at 28°C for the preparation of the next step.

### Extraction of dsRNA

All fungal strains were transferred to in the potato dextrose liquid medium (PD), and placed on the shaker at 200 r/min and

cultured at 28°C, after 2 days, collecting the mycelium for dsRNA extraction. DsRNA was extracted and purified from the mycelia by using CF-11 cellulose column chromatography [15]. Then, the samples were digested by RNase free DNase I (TaKaRa, Dalian, China) and S1 Nuclease (TaKaRa, Dalian, China) following the

manufacturer's instructions to remove any DNA and ssRNA contamination [13]. At last, electrophoresises of the samples were run in 1% agarose gel, and the dsRNA was detected by ultraviolet transillumination.

**Supplemental Table S1:** The information of strains of *Trichoderma* spp. collected from Xinjiang, Inner Mongolia, Jilin and Heilongjiang provinces of China.

Strain Number	Strain Name	Region
CTCCSJ-G-QT40112	<i>Trichoderma.cf. harzianum</i>	Inner Mongolia
CTCCSJ-G-QT40125	<i>Trichoderma longibrachiatum</i>	Inner Mongolia
CTCCSJ-F-KY40127	<i>Trichoderma.cf. harzianum</i>	Inner Mongolia
CTCCSJ-G-QT40130	<i>Trichoderma.cf. harzianum</i>	Inner Mongolia
CTCCSJ-F-KY40144	<i>Trichoderma.cf. harzianum</i>	Inner Mongolia
CTCCSJ-G-QT40154	<i>Trichoderma longibrachiatum</i>	Inner Mongolia
CTCCSJ-F-KY40155	<i>Trichoderma longibrachiatum</i>	Inner Mongolia
CTCCSJ-F-ZY40169	<i>Trichoderma longibrachiatum</i>	Inner Mongolia
CTCCSJ-G-QT40173	<i>Trichoderma longibrachiatum</i>	Inner Mongolia
CTCCSJ-G-QT40176	<i>Trichoderma longibrachiatum</i>	Inner Mongolia
CTCCSJ-G-HB40189	<i>Trichoderma longibrachiatum</i>	Xinjiang
CTCCSJ-G-QT40199	<i>Trichoderma longibrachiatum</i>	Xinjiang
CTCCSJ-G-HB40203	<i>Trichoderma.cf. harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40206	<i>Trichoderma.cf. harzianum</i>	Inner Mongolia
CTCCSJ-G-DK40213	<i>Trichoderma longibrachiatum</i>	Inner Mongolia
CTCCSJ-G-HB40215	<i>Trichoderma longibrachiatum</i>	Inner Mongolia
CTCCSJ-G-JK40241	<i>Trichoderma longibrachiatum</i>	Xinjiang
CTCCSJ-F-ZY40246	<i>Trichoderma afroharzianum</i>	Xinjiang
CTCCSJ-F-ZY40250	<i>Trichoderma afroharzianum</i>	Xinjiang
CTCCSJ-F-ZY40255	<i>Trichoderma longibrachiatum</i>	Inner Mongolia
CTCCSJ-G-QT40264	<i>Trichoderma longibrachiatum</i>	Inner Mongolia
CTCCSJ-G-QT40272	<i>Trichoderma longibrachiatum</i>	Inner Mongolia
CTCCSJ-G-HB40273	<i>Trichoderma longibrachiatum</i>	Inner Mongolia
CTCCSJ-G-HB40277	<i>Trichoderma longibrachiatum</i>	Inner Mongolia
CTCCSJ-F-ZY40291	<i>Trichoderma afroharzianum</i>	Xinjiang
CTCCSJ-F-ZY40300	<i>Trichoderma afroharzianum</i>	Inner Mongolia
CTCCSJ-G-QT40303	<i>Trichoderma asperelloides</i>	Xinjiang
CTCCSJ-G-QT40320	<i>Trichoderma hamatum</i>	Inner Mongolia
CTCCSJ-G-QT40324	<i>Trichoderma koningiopsis</i>	Inner Mongolia
CTCCSJ-G-QT40330	<i>Trichoderma gamsii</i>	Inner Mongolia
CTCCSJ-G-JK40357	<i>Trichoderma longibrachiatum</i>	Xinjiang
CTCCSJ-G-DK40360	<i>Trichoderma afroharzianum</i>	Inner Mongolia
CTCCSJ-G-QT40366	<i>Trichoderma longibrachiatum</i>	Inner Mongolia
CTCCSJ-F-KY40368	<i>Trichoderma longibrachiatum</i>	Inner Mongolia
CTCCSJ-G-QT40388	<i>Trichoderma afroharzianum</i>	Inner Mongolia
CTCCSJ-G-HB40403	<i>Trichoderma viridescens</i>	Xinjiang
CTCCSJ-G-HB40405	<i>Trichoderma saturnisporum</i>	Inner Mongolia

CTCCSJ-G-QT40408	<i>Trichoderma longibrachiatum</i>	Inner Mongolia
CTCCSJ-G-JK40417	<i>Hypocrea semiorbis</i>	Xinjiang
CTCCSJ-G-HB40436	<i>Trichoderma atroviride</i>	Xinjiang
CTCCSJ-G-HB40441	<i>Trichoderma harzianum</i>	Xinjiang
CTCCSJ-G-HB40456	<i>Trichoderma gamsii</i>	Xinjiang
CTCCSJ-G-HB40457	<i>Trichoderma guizhouense</i>	Inner Mongolia
CTCCSJ-G-HB40459	<i>Trichoderma.cf. harzianum</i>	Inner Mongolia
CTCCSJ-G-QT40460	<i>Trichoderma.cf. harzianum</i>	Inner Mongolia
CTCCSJ-G-QT40465	<i>Trichoderma.cf. harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40470	<i>Trichoderma.cf. harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40477	<i>Trichoderma.cf. harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40480	<i>Trichoderma.cf. harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40483	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40485	<i>Trichoderma koningiopsis</i>	Inner Mongolia
CTCCSJ-G-HB40491	<i>Trichoderma.cf. harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40502	<i>Trichoderma pleurotum</i>	Xinjiang
CTCCSJ-F-ZY40503	<i>Trichoderma citrinoviride</i>	Xinjiang
CTCCSJ-G-QT40507	<i>Hypocrea pseudoharzianum</i>	Xinjiang
CTCCSJ-G-HB40517	<i>Trichoderma citrinoviride</i>	Xinjiang
CTCCSJ-G-HB40519	<i>Hypocrea pseudoharzianum</i>	Xinjiang
CTCCSJ-G-HB40536	<i>Trichoderma longibrachiatum</i>	Xinjiang
CTCCSJ-G-HB40537	<i>Trichoderma harzianum</i>	Xinjiang
CTCCSJ-F-ZY40538	<i>Trichoderma citrinoviride</i>	Xinjiang
CTCCSJ-F-QT40542	<i>Trichoderma rossicum</i>	Xinjiang
CTCCSJ-G-HB40550	<i>Trichoderma asperellum</i>	Xinjiang
CTCCSJ-G-DK40556	<i>Trichoderma harzianum</i>	Xinjiang
CTCCSJ-G-DK40558	<i>Trichoderma harzianum</i>	Xinjiang
CTCCSJ-G-DK40579	<i>Trichoderma harzianum</i>	Xinjiang
CTCCSJ-G-QT40623	<i>Trichoderma harzianum</i>	Xinjiang
CTCCSJ-G-QT40624	<i>Trichoderma harzianum</i>	Xinjiang
CTCCSJ-F-KZ40639	<i>Trichoderma paraviridescens</i>	Xinjiang
CTCCSJ-F-KZ40642	<i>Trichoderma harzianum</i>	Xinjiang
CTCCSJ-G-JK40646	<i>Trichoderma harzianum</i>	Xinjiang
CTCCSJ-F-KZ40649	<i>Trichoderma harzianum</i>	Xinjiang
CTCCSJ-F-KZ40651	<i>Trichoderma harzianum</i>	Xinjiang
CTCCSJ-G-HB40673	<i>Trichoderma harzianum</i>	Xinjiang
CTCCSJ-F-KZ40703	<i>Trichoderma citrinoviride</i>	Xinjiang
CTCCSJ-G-HB40713	<i>Trichoderma rossicum</i>	Xinjiang
CTCCSJ-F-KZ40769	<i>Trichoderma paraviridescens</i>	Xinjiang
CTCCSJ-F-KZ40782	<i>Trichoderma harzianum</i>	Xinjiang
CTCCSJ-F-KZ40809	<i>Trichoderma harzianum</i>	Xinjiang
CTCCSJ-F-KZ40810	<i>Trichoderma harzianum</i>	Xinjiang
CTCCSJ-F-KZ40812	<i>Trichoderma paraviridescens</i>	Xinjiang
CTCCSJ-G-QT40814	<i>Trichoderma paraviridescens</i>	Xinjiang

CTCCSJ-F-KZ40816	<i>Trichoderma paraviridescens</i>	Xinjiang
CTCCSJ-F-KZ40827	<i>Trichoderma citrinoviride</i>	Xinjiang
CTCCSJ-G-QT40835	<i>Trichoderma asperellum</i>	Xinjiang
CTCCSJ-G-HB40862	<i>Trichoderma polysporum</i>	Xinjiang
CTCCSJ-G-HB40878	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-QT40900	<i>Trichoderma asperelloides</i>	Inner Mongolia
CTCCSJ-G-QT40916	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40920	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40921	<i>Trichoderma asperelloides</i>	Inner Mongolia
CTCCSJ-G-HB40925	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40927	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40929	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40930	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40932	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40934	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40939	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40940	<i>Trichoderma asperelloides</i>	Inner Mongolia
CTCCSJ-G-HB40941	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40946	<i>Trichoderma asperelloides</i>	Inner Mongolia
CTCCSJ-G-HB40957	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-F-KY40961	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-JK40972	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-JK40974	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40979	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-JK40980	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40987	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-HB40988	<i>Trichoderma longibrachiatum</i>	Inner Mongolia
CTCCSJ-G-HB40989	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-QT40994	<i>Trichoderma atroviride</i>	Heilongjiang
CTCCSJ-G-DK41012	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-HB41023	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-HB41038	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-HB41039	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-HB41044	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-HB41045	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-F-KY41047	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-QT41050	<i>Trichoderma rossicum</i>	Inner Mongolia
CTCCSJ-G-HB41053	<i>Trichoderma harzianum</i>	Inner Mongolia
CTCCSJ-G-JK41060	<i>Trichoderma harzianum</i>	Inner Mongolia

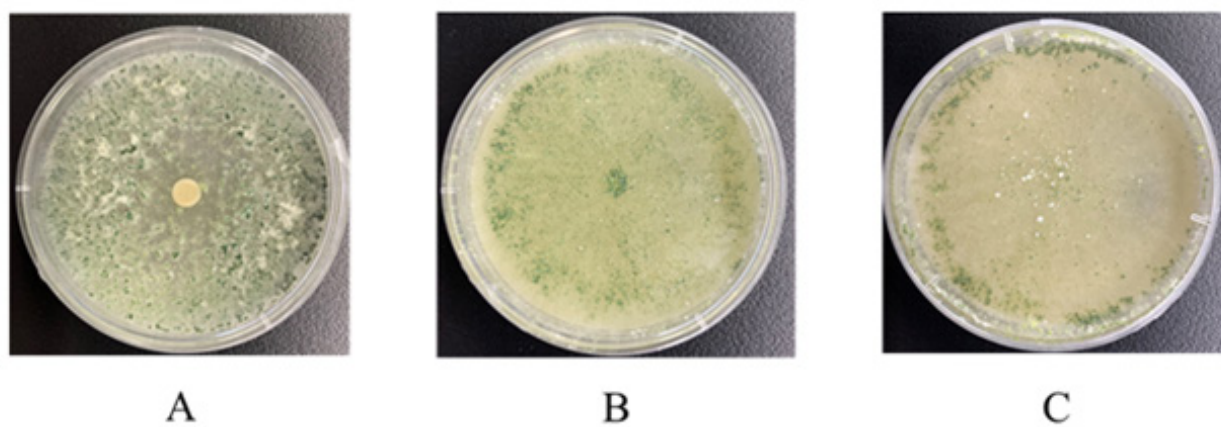
## Results

After 2 days' culture in PD, the mycelium of 120 strains of *T. spp.* were collected, then dsRNA of the 120 strains were extracted by the molecular biology methods. Though the existing of dsRNA

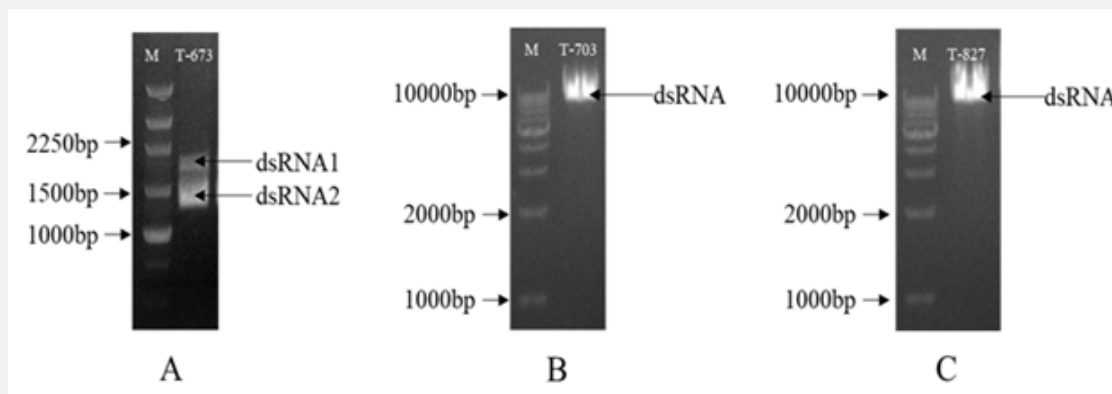
in the electrophoresis, the three *Trichoderma* strains with mycoviruses have been screened out. T673 was *T. harzianum*, T703 and T827 were *T. citrinoviride*, all of which were from Xinjiang province (Figures 1A, B, C). After dsRNA was extracted

from mycelium, two fragments of 1.5 and 1.7 KB were found in strain T673 (*T. harzianum*), then DNase I and S1 nuclease were used to confirm the type of nucleic acid, and dsRNAs were identified (Figure 2A). In the strain of T703 (*T. citrinoviride*), only

one fragment of 9 KB was found, and the dsRNA was digested by DNase I and S1 nuclease to confirm the properties of nucleic acid (Figure 2 B). With the same method, one dsRNA fragment of 9 KB was found in strain T827 (*T. citrinoviride*) (Figure 2C).



**Figure 1:** (A) The morphology of strain T673 (*T. harzianum*) cultured on PDA for 7 days at 28°C. (B) The morphology of strain T703 (*T. citrinoviride*) cultured on PDA for 7 days at 28°C. (C) The morphology of strain T827 (*T. citrinoviride*) cultured on PDA for 7 days at 28°C.



**Figure 2:** The electrophoresis of dsRNA mycovirus.

(A) Lane M: DNA marker (250 bp Ladder, TaKaRa); Line T673: dsRNA1 (approximately 1.7 kb) and dsRNA2 (approximately 1.5 kb) from the strain T673 of *T. harzianum* were digested by DNase I and S1 nuclease and run electrophoresis in 1% agarose gel. (B) Lane M: DNA marker (10 kb Ladder, TaKaRa); Line T703: dsRNA (approximately 9 kb) from strain T703 of *T. citrinoviride* was digested by DNase I and S1 and run electrophoresis in 1% agarose gel. (c) Lane M: DNA marker (10 kb Ladder, TaKaRa); Line T827: dsRNA (approximately 9 kb) from the strain T827 of *T. citrinoviride* was digested by DNase I and S1 nuclease and run electrophoresis in 1% agarose gel.

## Discussion

In this paper, we obtained three strains of mycoviruses from 144 strains of *T. spp.* isolated from the soil, which can preliminarily prove that mycovirus existed in *T. spp.*, and provided insight into how to locate and isolate mycoviruses from *T. spp.*. In the obvious researches, five genome sequences of mycoviruses from *T. spp.* were obtained, from which three strains were from *T. harzianum* [6-14]. In this issue, one mycovirus strain T-673 was explored from *T. harzianum*, another two strains from *T. citrinoviride*. At

present, for the investigation on DNA mycovirus was few in the nature, the DNA mycovirus from *T. spp.* has not been recovered [3]. All in all, the investigated dsRNA and DNA mycoviruses from *T. spp.* were few until now, which need to be explored more and more. In the future, nucleic acid type, the elimination, complete genome sequence, transmission mode and antagonistic effect on *Trichoderma* and biocontrol function of the mycovirus, as well as the interaction between fungi and mycoviruses, need to be revealed.

## Declarations

Ethics approval and consent to participate

This article does not contain any studies with human participants performed by any of the authors.

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## Competing interests

All of the authors have no conflicts of interest.

## Ethical approval

This article does not contain any studies with human participants performed by any of the authors.

## Consent for publication

Informed consent was obtained from all participants included in the study.

## Authors' contributions

RW performed the experiments, CL and ZT isolated *Trichoderma* spp., BW and XJ designed the study. All authors read and approved the final manuscript.

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