

ORIGINAL ARTICLE

Antifungal activity of several isolates of *Trichoderma* against *Cladosporium* and *Botrytis*

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Trichoderma isolates (SSBGT07, SSBGT08, SSBGT09, SSBGT10) were isolated from the soil samples of the South-Siberian Botanical Garden and identified using morphological observation and ITS region analysis as *Trichoderma harzianum*, *T. asperellum*, *T. ghanense*, and *T. longibranchiatum*. Antagonistic activity against *Cladosporium* sp. and *Botrytis* sp. was evaluated *in vitro*. All isolates showed antagonistic effect by competition against *Cladosporium* sp. *T. asperellum* and *T. longibranchiatum* showed antagonism against *Botrytis* sp. All isolates showed hyper sporulation on the sclerotia of *Botrytis* sp. (except the *T. ghanense*) and colonies of the *Cladosporium* sp. Our study provides new isolates that affect the *Cladosporium* sp. and *Botrytis* sp.

Key words: antifungal activity; *Botrytis*; *Cladosporium*; fungi; PCR; *Trichoderma*

Introduction

Trichoderma species are generally abundant on decaying wood and in soil because of their success in various heterotrophic interactions, including decomposition, parasitism, and even opportunistic endophytism (Druzhinina et al., 2006). *Trichoderma* spp. have important applications in industry and agrotechnology. Many species are bio control agents of plant pathogenic fungi, plant growth stimulators, destructors and antibiotic producers. *Trichoderma* plays a major role in controlling of various plant diseases. *Trichoderma* is widely represented in root, soil and foliar environments and used as a biofertilizer because of its ability to establish mycorrhiza-like association with plants. *Trichoderma* can decompose the crop residues into good quality compost not only in pit level but *in situ* also, which will improve organic matter along with macro, micronutrients, physicochemical and biological conditions of soil (Saba et al., 2012; Sharma et al., 2012). Many species of *Trichoderma* can act as bio-remediants and accumulate heavy metals from the environment (Hoseinzadeh et al., 2017). This property shows the potential for urban use. But undoubtedly one of the main features is the anti-fungal activity.

Materials and methods

Different *Trichoderma* species were isolated from soil probe of South-Siberian Botanical Garden. Isolate of *Trichoderma* species was isolated and identified on potato dextrose agar (PDA). Identification was carried out morphologically and by PCR analysis. *Botrytis* sp. and *Cladosporium* sp. were isolated from infectious plants. For isolate, a portion of a colony was scraped from the agar plates and transferred into a 1.5-ml Eppendorf tube. DNA was isolated by *DiamondDNA* kit (ABT LLC., Russia). The primers ITS1 5'- TCCGTAGGTGAACCTGCGG -3', ITS4 5'- TCCTCCGCTTATTGATATGC -3' were used for amplification (White et al., 1990). PCR(s) were carried out in 25 µL reaction mix which included 5 ng DNA, 2.5 µL 10x PCR buffer and 25 mM MgCl₂ (Sibenzyme Llc., Russia), 1 µL 5mM of mix dNTPs (Medigen Llc., Russia), 1 µL of each 10 mM primer and 1 unit Taq DNA polymerase (Sibenzyme Llc., Russia) in the *MyCycler* thermal cycler (Bio-Rad, USA) using protocol: 94.0 °C for 5 min. [94.0 °C for 30 sec., 56.0 °C for 30 sec., 72 °C for 1 min.]x35, 72.0 °C for 5 min., 4.0 °C until the end of the process. PCR products were then purified using spin column. Sequencing by Sanger was conducted in the Syntol LLC., Russia. The sequences of ITS (MG815133–MG815136) obtained in this study have been deposited in GenBank. Similarity checks were done at NCBI website.

For further analysis, sequences of ITS of closely related sequences were downloaded from NCBI. Alignment was done using *Muscle*, *MEGA 7.0* (Edgar, 2004). Phylogenetic analysis was done using *Mr. Bayes 3.2.6* with 1000000 MCMC and 100000 Burn-in period (Huelsenbeck, Ronquist, 2001).

Results and discussion

Phylogenetic analysis using ITS region recovered the fungus in a good-supported (0.99 and higher) clade with isolates of *Trichoderma asperellum* SSBGT07 (KP747443.1), *T. harzianum* SSBGT10 (MG372162), *T. longibrachiatum* SSBGT09 (MG372138.1), and *T. ghanense* SSBGT08 (KP419976.1). The sequence of *Protocrea farinosa* (DQ835506.1) was used as outgroup (Fig. 1).

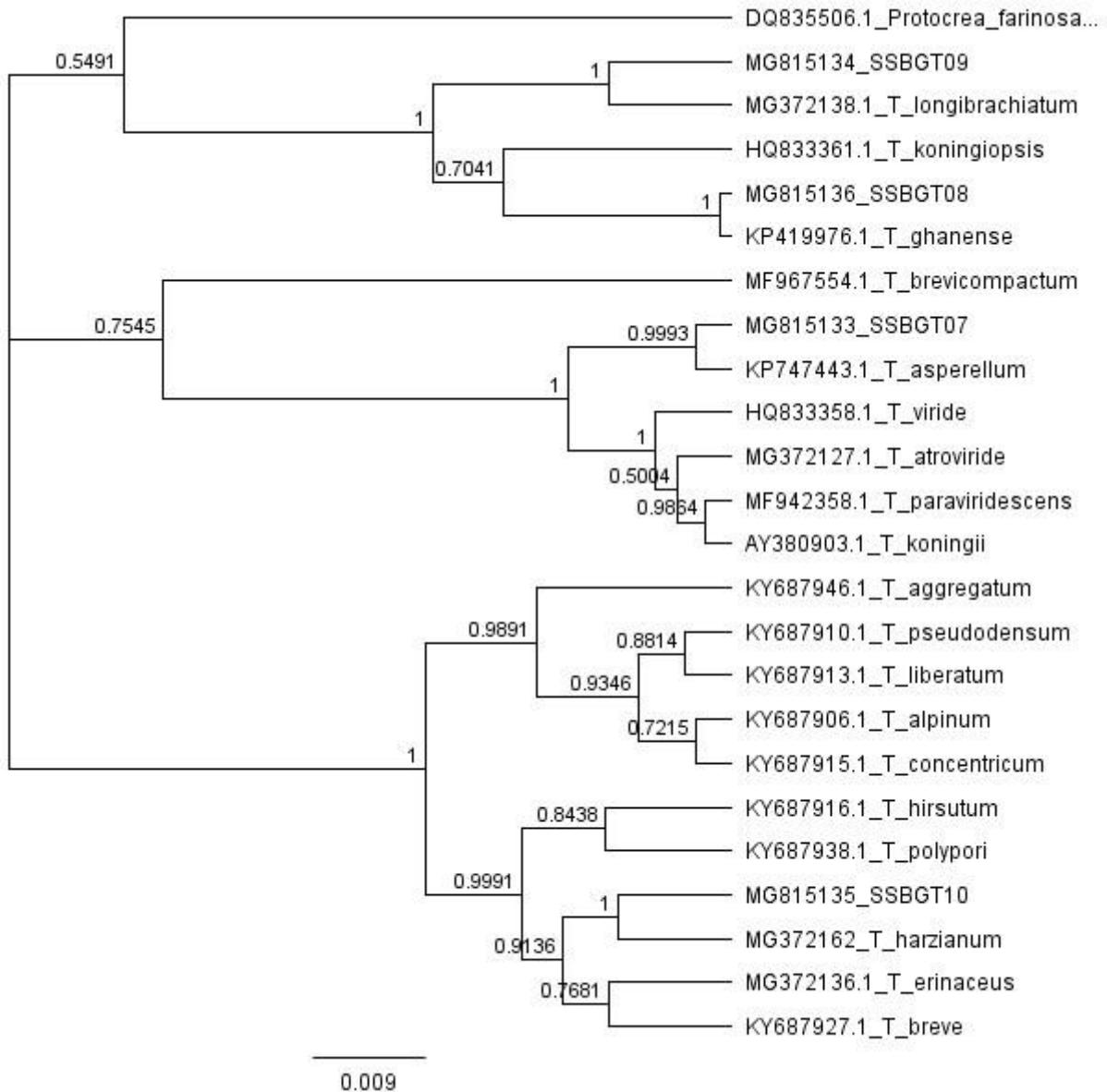


Fig. 1. Phylogenetic analysis based on ITS region of the *Trichoderma* isolates SSBGT07, SSBGT08, SSBGT09, SSBGT10.

The isolate showed a good growth rate on the PDA agar. Mature conidia were formed in 5-7 days after inoculation. For *T. asperellum*, we noted a high level of variability of colony morphology. *T. asperellum*, *T. longibrachiatum*, *T. harzianum* were characterized by rapid growth, hyper sporulation, covered the entire petri dish and stopped the development of *Cladosporium* sp. *T. ghanense* grew more slowly and less occupied the surface of the Petri dish, but it also had a hyper sporulation in places of conidia congestion of the *Cladosporium* sp.

Activity against *Botrytis* sp. for all isolates, was observed through the formation of an intermediate band without growth between colonies. The minimum activity and growth rate were observed in the *T. ghanense*. Maximum sporulation on sclerotia was observed for the *T. longibrachiatum* and *T. harzianum*. Furthermore, on day 12 after inoculation, *T. harzianum* showed growth and formation of conidia in the growth area of *Botrytis* sp. We also noted inhibition by *T. harzianum* of sclerotic formations of *Botrytis* sp. (Fig. 2).

The use of *Trichoderma* has a high potential in plant protection. Several authors reported that the antagonistic fungi like *Trichoderma harzianum*, *T. viride*, and *Gliocladium virens* inhibited the growth of pathogens by mycoparasitism and antibiosis (Mukherjee et al., 2002; Zeilinger, Omann, 2007). *Trichoderma* as bio-agents suppressed colony growth of *Fusarium oxysporum* (Shahid et al., 2014).

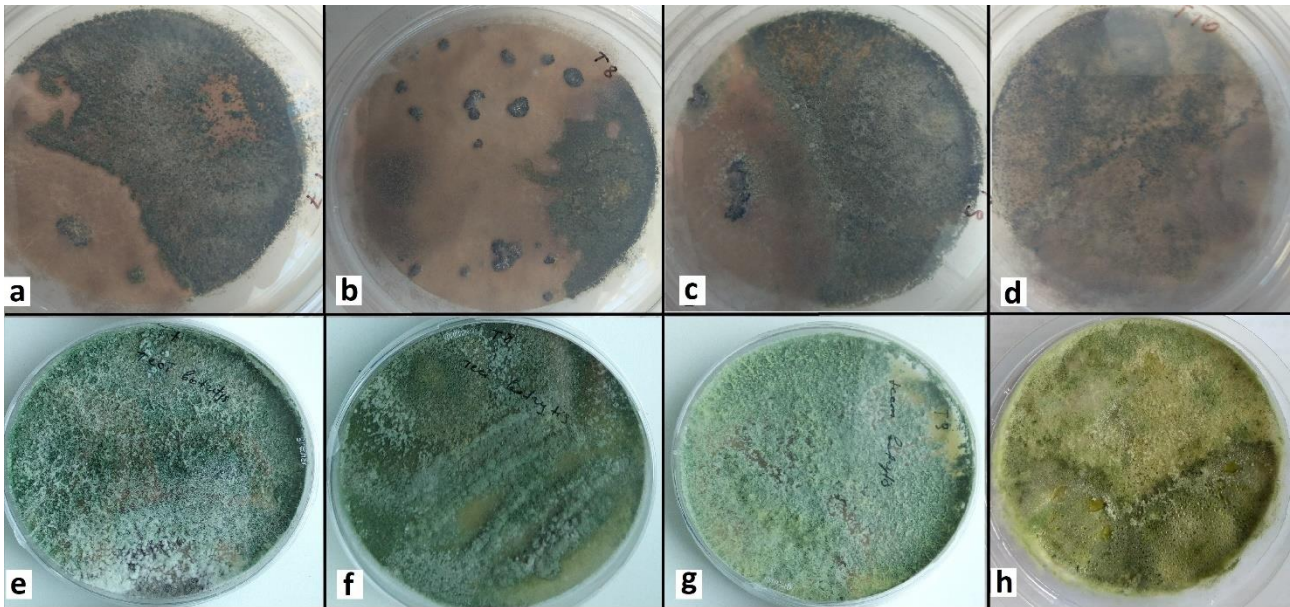


Fig. 2. Antagonistic effect of *Trichoderma* species against *Botrytis* sp.: a. *T. asperellum*; b. *T. ghanense*; c. *T. longibranchiatum*; d. *T. harzianum*. Antagonistic effect of *Trichoderma* species against *Cladosporium* sp.: e. *T. asperellum*; f. *T. ghanense*; g. *T. longibranchiatum*; h. *T. harzianum*. Hyper sporulation by *Trichoderma* is seen on the colonies of *Cladosporium* sp. (especially on conidia congestion) and sclerotia of *Botrytis* sp.

Another investigation revealed a high level of hyperparasitism of *Trichoderma koningiopsis* against *Macrophomina phaseolina* (Mendoza et al., 2015). *T. hamatum* and *T. pseudokonigii* showed a very high antagonistic level against *Aspergillus flavus* (Thanh et al., 2014). According to some reports, *Trichoderma* is an antagonist of such species as the *Botrytis cinerea*, *Penicillium crustosum*, *Alternaria alternata*, *Fusarium solani*, and *Aspergillus nidulans* (Belete et al., 2015; Rios Velasco et al., 2016).

The potential of various species of *Trichoderma* is expanding with new research. The high level of polymorphism of the *Trichoderma* species opens great opportunities to select strains with new characteristics, thus, expanding the possibilities of *Trichoderma* species as a bioagent.

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