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Study on secondary metabolites of *Trichoderma atroviride* F742 and their role in antibiosis

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Abstract: Soil-borne pathogenic fungi responsible for serious damage in agriculture are widely distributed. Traditional approach to control these pathogens leads to the use of chemical fungicides, many of which have proven harmful side effects on the environment. Mycoparasitic fungi of the genus *Trichoderma* have been used for pathogens biocontrol as well as for their ability to promote plant growth. As increased mycoparasitic activity of mutant strain *Trichoderma atroviride* F742 has been observed, its use as a biocontrol agent might be considered. In this work, we focused on mycoparasitic activity of the strain *T. atroviride* F742 prepared by UV mutagenesis (from parental strain *T. atroviride* F534) and the physiological role of *T. atroviride* metabolites in antibiosis.
T. atroviride F742 shows remarkable mycoparasitic activity attacking and colonizing phytopathogens (*Alternaria alternata*, *Botrytis cinerea*, *Fusarium culmorum*). Its isolated metabolites inhibit the growth of bacteria, yeasts, and fungi *Candida albicans*, *A. alternata* and *F. culmorum*. As different expression patterns in the secondary metabolites production of ABC transporters have been observed, we suggest their role in transport of secondary metabolites produced by *T. atroviride* F742.

Keywords: *Trichoderma* sp., secondary metabolites, mycoparasitism, ABC transporters, antimicrobial potential of produced metabolites

Introduction

Soil pathogenic fungi can cause serious damage in agriculture (Yu et al., 2021). Traditional approach to pathogen eradication involves the use of chemical fungicides, which have often been shown to have harmful side effects on the environment. An attractive way of reducing the concentration of chemical fungicides in agriculture is their combination with biocontrol strains (Howell, 2003; Sood et al., 2020). One of the possibilities for biocontrol are mycoparasitic fungi of the genus *Trichoderma* (Mukherjee et al., 2012; Poveda et al., 2020) which, apart from their mycoparasitic activity, protect plants and promote their growth (Raaijmakers et al., 2009; Aime et al., 2013).
Trichoderma sp. **mycoparasitism** is a result of combined processes including the production of antifungal secondary metabolites (Dennis and Webster, 1971; Claydon et al., 1987; Schirmböck et al., 1994; Lorito et al., 1996; Vinale et al., 2008), competition of nutrients (Chet, 1987), changes in morphological structure such as coiling, curling, and formation of appresorium-like structures (Elad et al., 1983; Lu et al., 2004).
Fungal antibiosis is strongly connected to the secretion of enzymes, antibiotics, and various secondary metabolites. Studies on suppression of plant pathogens have identified many different molecules involved in this process (Vey et al., 2001; Benitez et al., 2004; Khan et al., 2020), including peptaibols, 6-pentyl-α-pyrone, viridin, gliovirin, glisoprenins, gliotoxin, and various others. Apart from being toxic to pathogens (Pascale et al., 2020; Raaijmakers et al., 2009), these compounds are also involved in the promotion of plant growth (Lee et al., 2016). **Polyketides,** including polyphenols, anthraquinones, macrolides, polyenes, enediynes and polyethers, represent a highly diverse group of molecules with carbon skeletons. Considering the structural and functional diversity of polyketides, their synthesis is a result of controlled assembly of acetate and propionate. *Trichoderma* sp. genome contains more orthologues of polyketide synthase (PKS) genes with polyketide playing an important role in the fungus biology. Genomes of *T. atroviride* and *T. virens* contain 18 PKSs while the *T. reesei* genome has 11 PKSs (Baker et al., 2012). However, insufficient knowledge of secondary metabolites and their involvement in the antagonism has to be considered. A possible role in the antagonism is indicated by the expression of two *T. atroviride* PKS genes during the confrontation with plant pathogen *Rhizoctonia solani* (Mukherjee et al., 2012; Roveda et al., 2020).
While the production and secretion of antimicrobial compounds is an important factor of fungal antagonism, natural ability to protect themselves against toxins must be considered. The expression of ABC transporters enables protection against various phytopathogen toxins. Their detoxification

function is well known, but their other physiological functions need to be further investigated. It is known that ABC transporters are not restricted to one particular substrate/s (Higgins, 2001). This diversity of substance specificity is reflected in the variety of physiological roles played by ABC transporters in the cell. There is a significant role of each ABC transporter in the export of a variety of secondary metabolites (including those participating in antagonistic interactions), waste products or toxins from the cell. For example, deletion mutants of *T. atroviride Taabc*2 (G family ABC transporter) showed decreased resistance to fungal inhibitory compounds and their ability to protect tomato plants from *Pythium ultimum* and *R. solani* was impaired (Alfiky et al., 2021; Ruocco et al., 2009). In addition, null mutation of gene *CrabcG*5 *Clonostachys rosea* decreased the antagonism action against *Fusarium graminearum*, which resulted in unsuccessful protection of barley seedlings from foot rot diseases (Dubey et al., 2014a).

In this work, we focused on mycoparasitic activity of the strain *T. atroviride* F742 prepared by UV mutagenesis in confrontation with three different phytopathogens (*Alternaria alternata*, *Botrytis cinerea*, *Fusarium culmorum*). Secondary metabolites were isolated to determine their role in antibiosis and to design their possible transport mechanism via ABC transporters.

Materials and methods

Microorganisms and culture conditions

Fungal strains of *T. atroviride* CCM F534, *T. atroviride* CCM F742, *A. alternata* CCM F128, *B. cinerea* CCM F16, and *F. culmorum* CCM F21 were maintained on potato/dextrose/agar (PDA, Biolife, Italy) plates at 25 °C prior to their use in the experimental procedures. Bacteria (*Escherichia coli* CCM 3988, *Pseudomonas aeruginosa* CCM 1955, *Proteus vulgaris* CCM 1955, *Enterobacter cloacae* CCM 1903, *Staphylococcus aureus* CCM 3953, *S. epidermidis* CCM 4505) were maintained on Mueller-Hinton/Agar (MHA, Biolife, Italy) plates at 37 °C prior to their use in the experiments. All fungal and bacterial strains were obtained from the Czech Collection of Microorganisms. Model yeast *Candida albicans* SC 5314/ATCC MYA-2876 and *Candida parapsilosis* ATCC 22019 were obtained from the American Type Culture Collection and were maintained on Sabouraud agar (SDA, Biolife Italy) at 30 °C prior to their use in the experiments.

Dual culture assay

To examine the antagonistic activity of *T. atroviride* F742 against three phytopathogenic fungi, a dual culture assay was performed. Mycelial discs (5 mm diam.) of both mycoparasite (*T. atroviride* F742) and phytopathogen cultures were taken from the edge of actively growing colonies (48 h) and placed on the opposite site of fresh PDA 1.5 cm from the edge of the Petri dish. Fungal colonies were incubated for ten days in dual culture. After the confrontation of filamentous fungi, the antagonistic interaction was evaluated in terms of the mycoparasite growth over the phytopathogen (i), conidiation of the mycoparasitic fungus (ii) as well as conidiation on the phytopathogen colony (iii), according to Bell et al. (1982). To determine the microbistatic or microbicidal effect on phytopathogens, discs were taken from the confrontation zone after ten days of cultivation and placed on fresh PDA growth medium with benomyl (5 $\mu g \cdot mL^{-1}$, blocking any *Trichoderma* sp. growth, but not the phytopathogen; MIC of benomyl = 1 $\mu g \cdot mL^{-1}$ with microbistatic effect on *Trichoderma* sp., data not shown). If the growth of the phytopathogen was not observed after four days of cultivation, a disc of mycelium was taken and placed into 1 mL of Tween 80 solution, briefly shaken on vortex to release conidia and 250 µL was spread onto PDA plates. After 48 hours, the colonies of fungi grown from conidia were counted.

Transcriptomic analysis of ABC transporters during the production of secondary metabolites

During the secondary metabolites production on PDA growth medium covered with cellophane, the mycelium was harvested after 1st, 2nd and 3rd week. After being frozen in liquid nitrogen, 100 mg of mycelium was homogenized to powder. RNA was then extracted using the TRI reagent method (Sigma Aldrich, USA). After DNAase treatment (Qiaqen, USA) RNA was used for cDNA synthesis with the Masterscript RT-PCR System (5 Prime, USA). cDNA was subsequently used for the PCR reaction (PerfectTaq Plus DNA Polymerase kit, 5 Prime, Germany) with cycling procedure as follows: initial denaturation at 94 °C for 2 min; 30 cycles of denaturation at 94 °C for 30 s; appropriate annealing temperature for each primer set (Tab. 1) for 30 s; synthesis at 68 °C for 1 min; and further final synthesis of 10 min at 68 °C. The obtained PCR products were subjected to electrophoresis in 1.3 % agarose gel and visually shown under UV light (UVP Cambridge, UK).

Secondary metabolites extraction

Secondary metabolites *T. atroviride* F742 were isolated after cultivation on PDA growth medium inoculated on cellophane and cultivated for 21 days. Metabolites produced in the growth medium were then extracted with ethyl acetate. The organic fraction was dried

(Na_2SO_4), vaccum-concentrated at 40 °C to get a solid/oil-like residue, which was then diluted in 1 mL of ethyl acetate. The metabolites were visualized using thin layer chromatography (200 µm thick aluminum TLC plates, Silica gel 60F254; Merck Schüchardt, Germany), with 5 µL of metabolite extract. The plates were developed in a solvent system containing benzene and acetone (3:1). The secondary metabolites were then visualized in a visible spectrum and under UV light. Metabolite extract was then transferred into a microtube, dried under reduced pressure at 40 °C, weighed and diluted in 1 mL of DMSO, and used for the detection of antimicrobial activity.

Antimicrobial activity of isolated secondary metabolites

Sensitivity of bacterial strains and yeasts was determined by the broth micro-dilution method (CLSI, 1996; 2014). Antifungal activity (filamentous fungi) was evaluated by the macrodillution method (Dudová et al., 2002).

Biofilm formation in vitro

Biofilm formation was assayed by crystal violet staining in a 96-well polystyrene microtiter plate. In short, *C. albicans* was cultivated for 16 h (overnight inoculum) in Sabouraud broth (SB, Biolife Italy) supplemented with 2 % glucose under shaking (200 rpm). The culture was diluted to cell density of 10^7/mL into SB and 198 µL aliquots of fresh diluted cells were added into wells of a 96-flat bottom well polystyrene microplate (Sarstedt, Nümbrecht, Germany). For the biofilm inhibition assay, 2 µL of secondary metabolites (final concentration of 2.5—10 $\mu L \cdot mL^{-1}$) were added to 198 µL of inoculated SB medium. As the control, DMSO (1 %) treated cells were used. First, cells were cultivated without shaking at 37 °C for 2 h (phase of adherence). After 2 h, the culture medium was replaced by a fresh one (supplemented with metabolites), so only the cells that adhered to the plate surface formed the biofilm. The plates were then incubated without shaking at 37 °C for 48 h. The biofilm-coated wells were washed twice with 200 µL of PBS and then air dried for 15 min. Washed wells were stained with 110 µL of 0.4 % aqueous crystal violet solution for 15 min. Afterwards, each well was washed four times with 350 µL of sterile distilled water and immediately washed with 200 µL of 95 % ethanol. After 15 min of washing, 100 µL of the washing solution were transferred to a new well and the amount of the crystal violet stain in the washing solution was measured with a microtiter plate reader at 570 nm. Biofilm intensity was evaluated according to the observed absorbance values as weak, medium, or strong — [A570 <0.1—0.29> weak biofilm formation; A570 <0.3—0.5> medium biofilm formation; A570 >0.5 strong biofilm formation]. All experiments were performed in six parallel measurements.

Results

The model strain *T. atroviride* F742 was gained by UV mutagenesis from the parental strain *T. atroviride* F534 (Betina et al., 1986) and is characterized by brown conidia and the production of brown-red pigment (Fig. 1, Fig. 2) consisting of 1-acetyl-2,4,5,7-tetrahydroxyanthraquinone which is characterized by Rf = 0.32 (Betina et al., 1986).

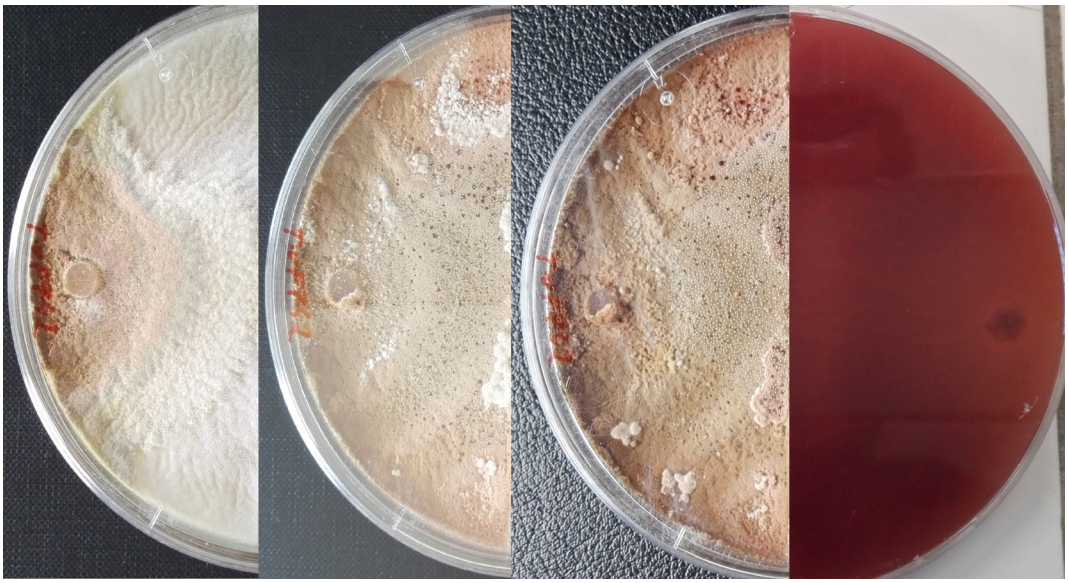

Fig. 1. Production of brown-red pigment during three weeks of *T. atroviride* F742 cultivation on PDA growth medium.

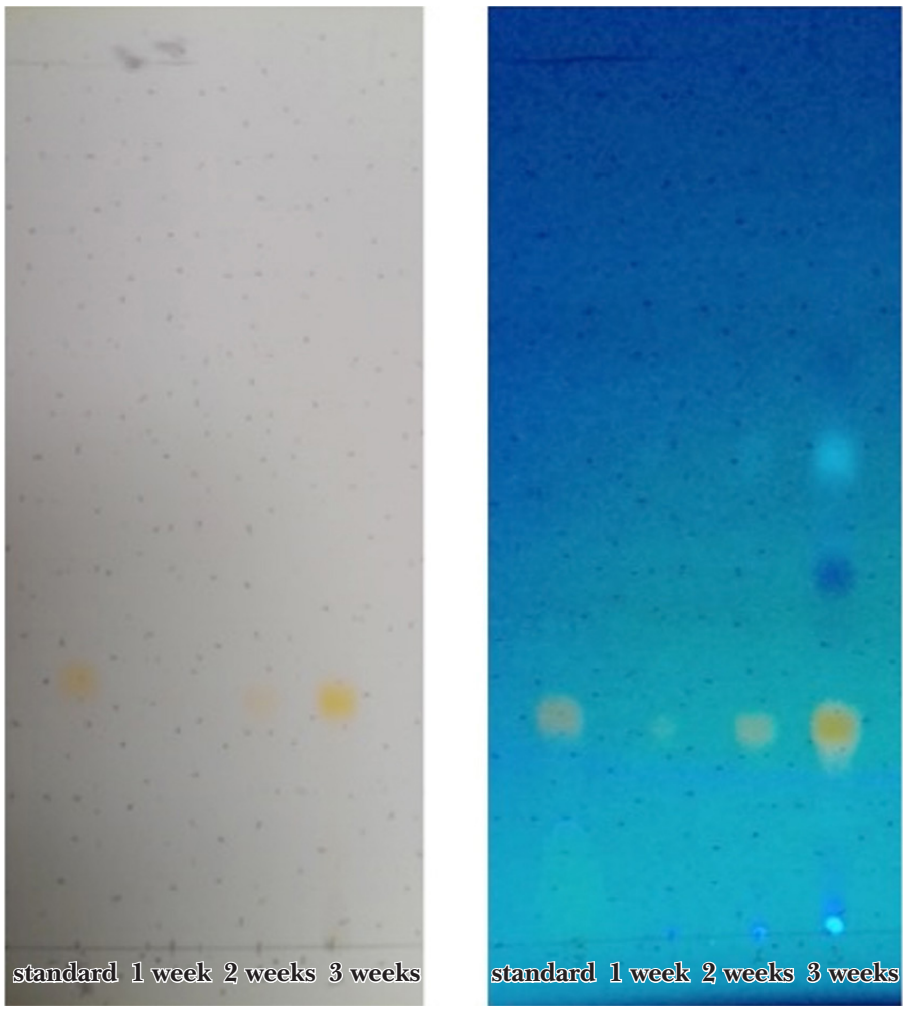

Fig. 2. Production of brown-red pigment — 1-acetyl-2,4,5,7-tetrahydroxyanthraquinone and other secondary metabolites, by *Trichoderma atroviride* F742 on PDA growth medium visualized on TLC plates under VIS light (left) and UV light (right).

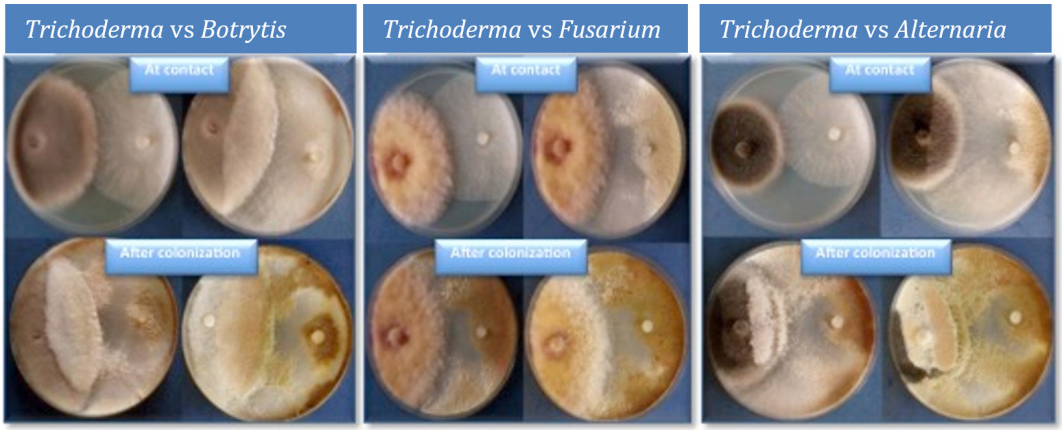

Fig. 3. Mycoparasitic activity of *Trichoderma atroviride* F742 against phytopathogens. At contact — the first picture shows the primary contact of model fungi, while the second picture shows slight overgrowth of phytopathogen. After colonization — the first picture shows overgrowth of the phytopathogen and the second picture manifest full colonization of model fungus.

In this work, increased mycoparasitic activity of *T.atroviride* F742 has been observed when compared to that of the parental strain *T. atroviride* F534 (Fig. 3). Parental strain *T. atroviride* F534 is able to inhibit the growth of all model phytopathogens but it is not able to overgrow them. On the other hand,

as it is shown in Fig. 3, *T. atroviride* F742 is able to attack and colonize all model phytopathogens.

The results of mycoparasitic interaction and conidiation of the mycoparasite on the phytopathogen colony are summarized in Tab. 1. Phytopathogens were intensively colonized by *T. atroviride* F742, with intense conidiation on the phytopathogen surface. The effect of mycoparasite on phytopathogens was estimated as lethal — there was no growth of phytopathogen observed after the mycelium disk was taken from the overgrown area and placed on the PDA growth medium supplemented with benomyl (5 $\mu g \cdot mL^{-1}$). The benomyl concentration of 5 $\mu g \cdot mL^{-1}$ is fungistatic for *Trichoderma* sp. (including *T. atroviride* F742) but does not inhibit the growth of the phytopathogen. The fungistatic benomyl concentration for phytopathogens is above 10 $\mu g \cdot mL^{-1}$ (Kubová et al., 2022). The phenotype difference between the mutant and the parental strain is mainly in the different conidial color followed by the spectra of secondary metabolites (Fig. 2) with Rf factors of 0.32 (1-acetyl-2,4,5,7-tetrahydroxyanthraquinone), 0.4 and 0.516. In this work, the physiological role of secondary metabolites reflecting their role in antibiosis as well as the potential transport system for secondary metabolites, the ABC transporters, were investigated.

The transcriptomic profile of ABC transporters type G (Taabc2—Taabc9) and MDR transporters (MDR1—MDR8) (Kubová et al., 2022) were determined together with anthraquinone and other secondary metabolites production. Induction of PDR genes expression was observed for Taabc9, Taabc2, and Taabc7 after the first week of cultivation (Fig. 5), while the expression of MDR genes MDR6, MDR4, MDR2, MDR1, and MDR5 (Fig. 6) was observed at a later exponential phase, the highest gene expression was observed during the stationary phase (after 2nd and 3rd week), which correlates with the production of secondary metabolites (Fig. 2).

As the synergy of the produced secondary metabolites may contribute to antibiosis with other microorganisms, antimicrobial potential of *T. atroviride* F742 metabolites is also shown in our study. Antimicrobial activity was assayed on model bacteria, yeasts, and phytopathogenic fungi. Inhibition of *E. coli*, *S. aureus* and *S. epidermidis* were observed (Tab. 2), with the highest susceptibility of *E. coli* (58 % growth inhibition). Growth inhibition of three phytopathogens, *B. cinerea*, *A. alternata,* and *F. culmorum,* in the presence of isolated metabolites was monitored via the macrodilution method. The growth of *B. cinerea* was not affected, but partial inhibition (30 %) of *A. alternata* and *F. culmorum* was observed after three days. Model yeast *C. albicans* was also inhibited (40 %) by the sum of metabolites (Tab. 3). As candidiasis is often followed by biofilm formation, the potential of *C. albicans* biofilm eradication by the sum of secondary metabolites was studied. The biofilm was partially eradicated by the sum of secondary metabolites as shown in Tab. 4. In comparison with the control (no metabolites,

Tab. 1. Mycoparasitic activity of *Trichoderma atroviride* F534 and *Trichoderma atroviride* F742 in dual culture assay.

Strain	*B. cinerea*	*A. alternata*	*F. culmorum*
Pathogen colonization[a]			
T. atroviride F534	1	1	1
***T. atroviride* F742**	3	3	3
Conidiation on plate[b]			
T. atroviride F534	1	1	1
***T. atroviride* F742**	3	3	3
Conidiation on pathogen colony[c]			
T. atroviride F534	1	1	1
***T. atroviride* F742**	3	3	3

[a]0 — Mycoparasite does not grow over the phytopathogen colony. 1 — mycoparasite partly grows over the phytopathogen colony (5—20 mm from the zone of contact of both micromycetes). 2 — mycoparasite grows over the phytopathogen colony (21—40 mm from the zone of contact). 3 — mycoparasite intensely colonizes the surface of the phytopathogen (41—60 mm from the zone of contact).

[b]0 — Mycoparasite does not form conidia outside the phytopathogen colonies. 1 — weak conidiation of the mycoparasite outside the phytopathogen colonies (10—100 conidia per cm^2), 2 — conidiation of the mycoparasite outside the phytopathogen colonies (10^3—10^4 conidia per cm^2), 3 — intense conidiation of the mycoparasite beyond the phytopathogen (10^5—10^6 conidia per cm^2).

[c]0 — Mycoparasite does not form conidia outside the phytopathogen colonies. 1 — weak conidiation of the mycoparasite outside the phytopathogen colonies (10—100 conidia per cm^2), 2 — conidiation of the mycoparasite outside the phytopathogen colonies (10^3—10^4 conidia per cm^2), 3 — intense conidiation of the mycoparasite beyond that of the phytopathogen (10^5—10^6 conidia per cm^2).

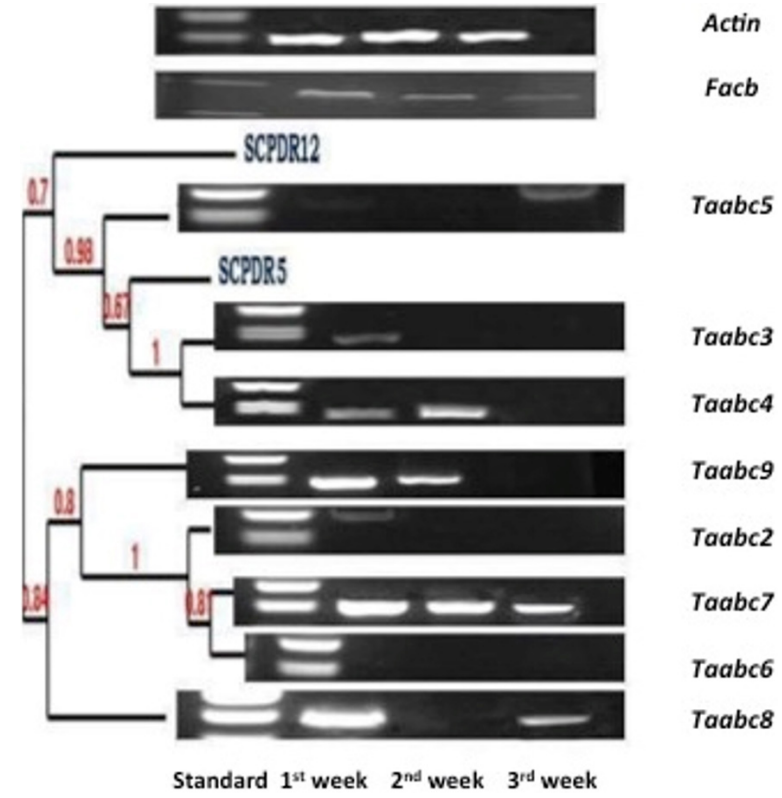

Fig. 5 Transcriptomic analysis of ABC transporter (ABC G-family) genes Taabc2—Taabc9 of *T. atroviride* F742 during three-week cultivation on PDA growth medium.

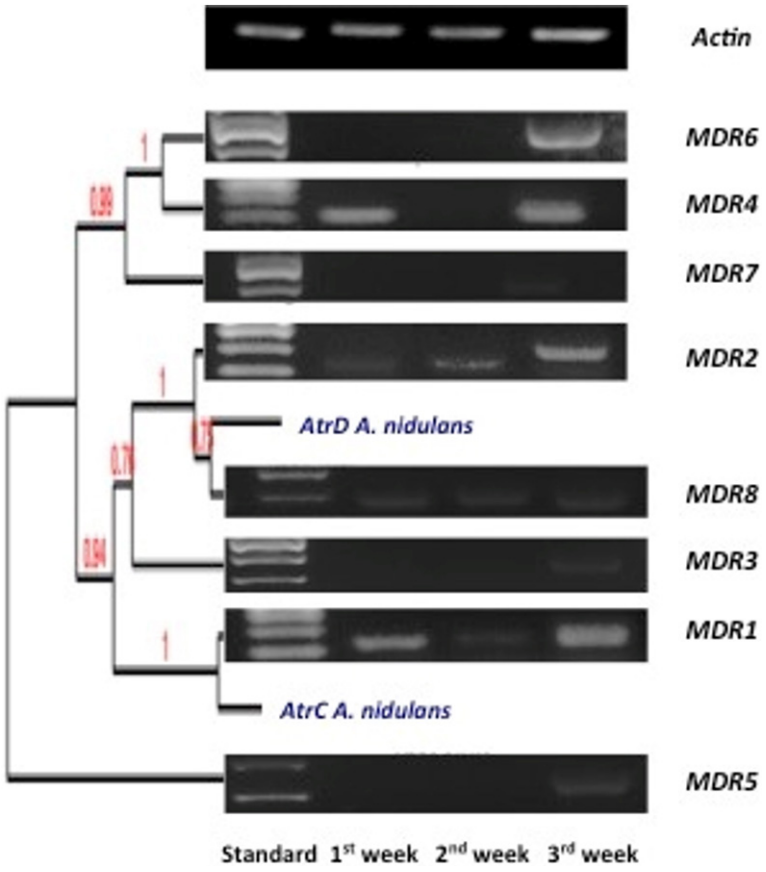

Fig. 6 Transcriptomic analysis of MDR transporter (ABC B-family) genes MDR1—MDR8 of *T. atroviride* F742 during three-week cultivation on PDA growth medium.

1 % DMSO), the intensity of biofilm formation decreased from strong to weak. Apart from their mycoparasitic activity, fungal strain *T. atroviride* F742 is also a potential source of new antimicrobial compounds as it is shown in this study.

Tab. 2. Antibacterial activity of extracted secondary metabolites (10 $\mu L \cdot mL^{-1}$).

Microorganism	Growth (%)
Escherichia coli CCM 3988	42
Proteus vulgaris CCM 3955	100
Enterobacter cloacae CCM 1903	100
Staphylococcus aureus CCM 3953	50
Staphylococcus epidermidis CCM 4505	65

Tab. 3. Antifungal activity of extracted secondary metabolites (10 $\mu L \cdot mL^{-1}$).

Microorganism	Growth (%)
Candida albicans SC 5314	60
Candida parapsilosis ATCC 22019	100
Alternaria alternata CCM F397	70
Fusarium culmorum CCM F21	70
Botrytis cinerea CCM F314	100

Tab. 4. Intensity of *C. albicans* biofilm in the presence of the sum of secondary metabolites of *T. atroviride* F742.

Metabolites	Intensity of formed biofilm	A_{570}
10 $\mu L \cdot mL^{-1}$	Weak	0.298
5 $\mu L \cdot mL^{-1}$	Medium	0.320
2.5 $\mu L \cdot mL^{-1}$	Medium	0.421
Control (1 % DMSO)	Strong	1.394

Discussion

Management of fungal diseases of food crops and fruits attracts more and more attention as soil-borne pathogenic fungi are widely distributed and are responsible for serious damages in agriculture (Rossman, 2009; Yu et al., 2021). Phytopathogenic fungi are not only responsible for plant diseases, but some representatives of *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp., are able to produce mycotoxins while growing on plants, which can seriously endanger consumers' health. The fight against phytopathogenic fungi is based on traditional approach — the use of chemical fungicides, which have been a very effective "weapon in the fight" against soil-borne fungal pathogens.

In recent years, environmental hazards caused by excessive use of pesticides have been widely discussed; therefore, scientists in the agricultural field are searching for alternative measures against pesticides. Furthermore, shortly after the introduction of site-specific fungicides into the plant protection management, resistance development in the pathogen populations was observed, leading to the loss of primary active fungicides (McBeath et al., 2010; Su et al., 2021). These findings strongly support more eco-friendly approach with the use of biological control as a potential tool to minimize the risk on human health and environment.

Mycoparasitic fungi of the genus *Trichoderma* have been used for biocontrol of pathogens like *Rhizoctonia solani, Sclerotium rolfsii* and *Botrytis cinerea* (Mukherjee et al., 2012; Roveda et al., 2020). Apart from their mycoparasitic activity, they protect plants by inducing a plant defensive system and promoting plant growth (Raaijmakers et al., 2009; Aime et al., 2013; Pascale et al., 2020). Our study has focused on the fungal strain *T. atroviride* F742 prepared by UV mutagenesis as a potential biocontrol agent for its use in environmentally friendly crops protection. In comparison with the parental strain, the characteristic feature of mutant strain *T. atroviride* F742 is brown-colored conidia followed by the production of brown-red pigment consisting of 1-acetyl-2,4,5,7-tetrahydroxyanthraquinone (Betina et al., 1986).

As increased mycoparasitic activity of *T. atroviride* F742 has been observed, its potential use in biocontrol is in play. The parental strain *T. atroviride* F534 was also able to inhibit the growth of phytopathogens but complete colonization has not been observed (Olejníkova et al., 2010). On the other hand, our mutant strain easily colonized all three model phytopathogens with intense conidiation on the surface of the phytopathogen colony. The effect of the mycoparasite on the phytopathogens was estimated as lethal — there was no growth observed after mycelium was taken from the overgrown area. As secondary metabolites play an important role in antibiosis, the physiological role of secondary metabolites of *T. atroviride* F742 was investigated. Also, fungal tools for transport of secondary metabolites were studied. Production of the most secondary metabolites of *Trichoderma* sp. is bound to the end of exponential and stationary growth phase, as documented by TLC (Fig. 2), which clearly shows that other secondary metabolites are produced together with anthraquinones. Anthraquinones are red-brown colored metabolites and as it is easy to follow their production; they have been chosen as an indicator of secondary metabolism initialization and progress. There is limited knowledge about the transport mechanisms of secondary metabolites

into the environment. Some studies indicate that the role of fungal ABC transporters is not limited only to the detoxification mechanisms.

Fungal MFS and ABC transporters are closely associated with the transport of host-specific or non-specific toxic metabolites by toxinogenic fungal phytopathogens (del Sobro et al., 2000; Jeandet et al., 2021). As mycotoxins produced by fungal strains are classified as secondary metabolites, the role of efflux pumps in secondary metabolites transport is thus confirmed. Moreover, the organization of gene clusters supports the physiological function of ABC transporters in secondary metabolite transport as these are clustered with gene coding PKS (polyketide synthesis), enzymes involved in synthesis of secondary metabolites (Andrade et al., 2000, Dohren et al., 2009). To determine the ABC transporters as transport systems for secondary metabolites produced by *Trichoderma* sp., the profile of ABC transporters — G family and ABC transporters of family B (MDR) were expressed in correlation with the production of anthraquinones and other secondary metabolites. Anthraquinones are in general synthesized from acetyl-CoA and malonyl-CoA via the Claisen condensation by PKS type I, which results in the formation of poly-β-ketide chain (Fig. 4). ABC transporters possess a binding site for Facb (transcription factor of acetate metabolism in fungi) as acetyl CoA is the precursor of polyketides and other secondary metabolites. The presence of the Facb site in regulation regions indicates their potential role in the transport of secondary metabolites. Bioinformatic analysis of the *Trichoderma* sp. genome (http://genome.jgi.doe.gov/Triat2/Triat2.home.html) has shown eight full and two half ABC-G transporters with high homology to yeast transporters PDR12 and PDR5 (*Saccharomyces cerevisiae*) and eight MDR transporters with high homology to AtrC and AtrD (*Aspergillus nidulans*) (Kubová et al., 2022). The transcriptomic profile of ABC transporters type G (Taabc2—Taabc9) and MDR transporters (MDR1—MDR8) genes were determined in correlation with anthraquinone and other secondary metabolites production. The expression of MDR genes MDR6, MDR4, MDR2, MDR1 and MDR5 (Fig. 6) was observed at later exponential phase (after two weeks) and at stationary phase (after three weeks) in correlation with secondary metabolites production. Thus, it can be assumed that MDR transporters have a role in the transport of secondary metabolites as their expression correlates with anthraquinones and other secondary metabolites production (Fig. 2).

Many microorganisms isolated from soil and plant-associated environments produce secondary metabolites that adversely affect the growth or metabolic activity of other microorganisms (Harman et al., 2021; Fravel, 1988). The production of secondary metabolites often favors its producer in the environment since many metabolites are toxic to other competing microorganisms in the same environment. Secondary metabolites are often the sources of rare antimicrobial active compounds (del Sorbo et al., 2000, Schoonbeek et al., 2002). While *Trichoderma* sp. are soil-born fungi related to plant rhizosphere, and their offensive and defensive strategies are well developed, they present a potential source of antimicrobial active compounds. In this work, we have shown how the whole sum of produced secondary metabolites contributes to antibiosis with other microorganisms via the antimicrobial potential of metabolites produces by *Trichoderma* sp. Antimicrobial effect on bacteria, yeasts, and phytopathogenic fungi has been observed. Antifungal potential was observed only when the whole sum of metabolites was present, which mirrors the synergism of metabolites produced in the environment. As it has been shown, *T. atroviride* F742 is a successful antagonistic

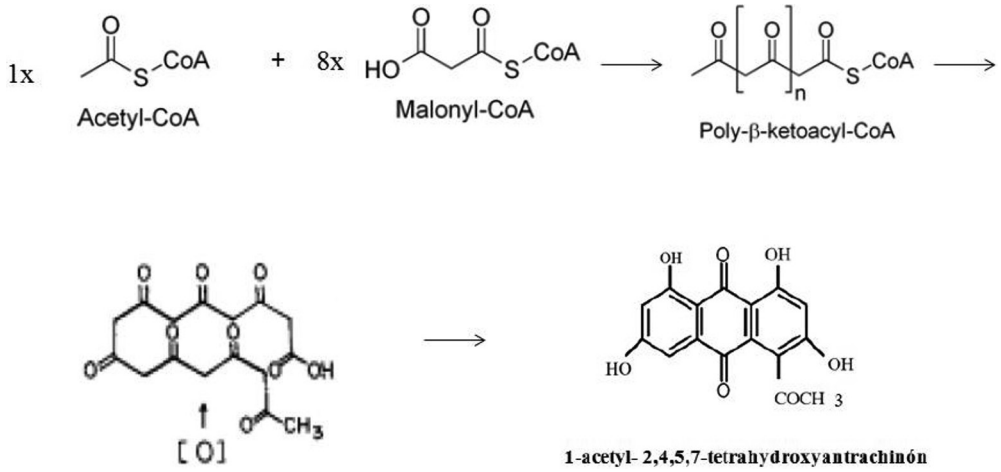

Fig. 4. Proposed synthesis of 1-acetyl-2,4,5,7-tetrahydroxyanthraquinone (Betina et al., 1986).

fungus and antifungal activity of its secondary metabolites can also contribute to its mycoparasitic properties by partial inhibition of phytopathogens growth. Its main contribution to mycoparasitic activity is considered to be the activity of hydrolytic enzymes degrading cell walls of phytopathogens (Druzhinina et al., 2011; Tyśkiewicz et al., 2022).

Conclusion

In this work, mycoparasitic activity of the strain *Trichoderma atroviride* F742 prepared by UV mutagenesis was confronted with three different phytopathogens. Specifically, secondary metabolites were isolated to determine its antimicrobial activity and possible transport system via ABC transporters MDR6, MDR4, MDR2, MDR1 and MDR5. The mutant strain *T. atroviride* F742 was able to attack and colonize all model phytopathogens with lethal effect on phytopathogenic fungi, while the parental strain *T. atroviride* F534 only inhibits the growth of model phytopathogens but is not able to colonize them. As different expression patterns of various ABC transporters have been observed, potential role of MDR in the transport of isolated secondary metabolites has been suggested. Antimicrobial testing of secondary metabolites showed the highest inhibition of *E. coli* and *S. aureus* among model bacteria. Phytopathogenic fungi (*A. alternata* and *F. culmorum*) were partially (30 %) inhibited suggesting that secondary metabolites produced by *T. atroviride* F742 can also contribute to its mycoparasitic activity. Even though the main contribution to their mycoparasitic activity is still considered to be the activity of hydrolytic enzymes degrading cell walls of phytopathogens, it is important to consider the contribution of other mechanisms, taking in account that mycoparasitic interaction is the synergy of several mechanisms. However, further and more detailed investigation is needed to study the increased mycoparasitic activity of *T. atroviride* F742.

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