



Research article

Diversity of *Sclerotium rolfsii* antagonist fungi isolated from soils of the rhizosphere of tomato crops and identification of some antifungal compounds



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ABSTRACT

Sclerotium rolfsii Sacc. the causative agent of white rot is one of the destructive pathogens of nightshade crops. In Côte d'Ivoire, this fungal pathogen constitutes a major constraint for the cultivation of tomato (*Solanum lycopersicum*) with 41.01% crop losses in humid forest areas. Controlling this fungus with synthetic chemicals can be effective, but harmful to human health and the environment. The use of biological control agents could be an alternative approach to control *S. rolfsii*. In this perspective, the objective of this work was to select fungi from the rhizosphere of tomato crops capable of inhibiting the growth of *S. rolfsii*. To do this, 153 fungi were isolated from the rhizosphere and from direct confrontation tests 10 fungi whose antagonistic power of *S. rolfsii* varied between 27 and 60% were selected. Molecular identification (ITS) of these antagonist fungi revealed that the isolates belonged to the genera *Talaromyces* sp. (n = 4), *Trichoderma* sp. (n = 3), *Penicillium* sp. (n = 2) and *Clonostachys* sp. (n = 1). Among these fungi, *Talaromyces purpureogenus* and *Talaromyces assiutensis* were able to diffuse compounds in agar capable of inhibiting the growth of *S. rolfsii*. The chemical study of these 2 fungi made it possible to identify mitorubrin and mitorubrinol produced by *T. purpureogenus* and spiculisporic acid produced by *T. assiutensis*. Mitorubrin and mitorubrinol had inhibitory activities of 100 and 70% at 10 mg/mL, respectively, whereas spiculisporic acid showed moderate inhibition of 38 at 20 mg/mL of the growth of *S. rolfsii*; however, its abundant production by the fungus could be an advantage in the control of this phytopathogen. Isolated from the same biotope as *S. rolfsii*, *T. purpureogenus* and *T. assiutensis* represent favorable candidates for the biological control against *S. rolfsii*.

1. Introduction

In agriculture, losses estimated at 20% of the annual yield are attributable to phytopathogenic fungi (Osman et al., 2017). The latter infect seeds, seedlings and mature or not mature plants causing many diseases such as damping-off, wilt (Nisha et al., 2009). *Sclerotium rolfsii* Sacc. causative agent of white rot is one of the destructive pathogens of plants worldwide (Le et al., 2012). This soil-borne fungus infects over 400

different plant species (Kim et al., 1999). In Côte d'Ivoire, this fungal pathogen constitutes a major constraint for the cultivation of tomatoes (*Solanum lycopersicum*) with 41.01% of crop losses in humid forest zones (Bolou et al., 2016a). The control of this fungal pathogen remains very difficult due to its survival structure (sclerotia) which remains viable in the soil for several years (Logan and Vos, 2011). The fight against this pathogenic fungus comes down to prophylactic measures and the use of synthetic pesticides (Bolou et al., 2015). However, the excessive use of synthetic

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fungicides leads to the development of resistant mutants and has harmful consequences on man and the environment. In Côte d'Ivoire, contamination of groundwater by organophosphate and organochlorine pesticides has been detected in agricultural regions where market gardening is practiced (Traoré et al., 2006). So many facts that require a sustainable and alternative management approach to fight against crop diseases.

Biological control by means of living microorganisms (biocontrol agent) could be a better approach to control *S. rolfsii* in order to reduce production losses and limit the use of synthetic fungicides. It consists of the use of microorganisms which either have a potential to inhibit the causal agent by different mechanisms of action, or an ability to increase the plant's defense mechanism. Microorganisms from the rhizosphere in areas where pest pressure is observed are the best candidate biocontrol agents. They are known for their ability to colonize the rhizosphere and the roots of plants, to co-evolve and sometimes control phytopathogenic microorganisms by various mechanisms including the production of bioactive metabolites. These bioactive metabolites are natural substances produced in response to interactions between protagonists (Galet, 2014; Belkadi and Koliai, 2016).

Songon, a town located in the south of Côte d'Ivoire with geographical coordinates 5° 18'59" North latitude and 4° 14'27" West longitude with an altitude of 10 m is a tomato production area where rife several pathogens including *S. rolfsii* (Bolou et al., 2016a; Kambiré et al., 2018). In addition to tomato plants, which suffer 83% of attacks from *S. rolfsii*, eggplant and pepper plants are also vulnerable. This showed an aggressiveness ranging from 52 to 90% against different varieties of pepper (Bolou et al., 2016a, 2016b). However, this proliferation of *S. rolfsii* can be inhibited by other microorganisms that coexist in the same habitat through different naturally developed mechanisms of action. The present work consists of the search for potential biocontrol agents against *S. rolfsii* isolated from soils under tomato crops.

2. Material and methods

2.1. Isolation of fungi from the soil

Rhizosphere soil samples were collected from six plots from different farmers. Sampling was carried out as described by Barillot et al. (2012). Briefly, using a sterile spatula, in each plot, a composite sample of 150 g of soil was made from 12 randomly selected subsamples in each plot. The composite samples were stored in sterile packaging bags (L x W: 200 × 145 mm). These were put into a cooler and then directly carried to the laboratory where they were stored at 4 °C. The various rhizospheric fungi were isolated by the suspension-dilution method (Rapilly 1968) on Potato Dextrose Agar (PDA) medium (Conda SA, Spain). For each soil sample, stock suspensions were prepared by mixing 1 g of soil into 9 mL of sterile distilled water. After vortexing for 5 min, a decimal dilution series is performed. The 10⁻³ to 10⁻⁵ dilutions were seeded on the PDA medium. Three tests are carried out for each of them. The dishes were incubated at 28 ± 1 °C for 7 days. The colonies obtained were subcultured on the PDA medium until pure cultures were obtained. All isolates were stored at -80 °C in 25% glycerol.

2.2. In vitro evaluation of the antifungal activity of soil fungi against *S. rolfsii*

The antifungal activity of fungi isolated from the soil against the phytopathogenic fungus *S. rolfsii* (provided by the Laboratory of Plant Physiology of the University Félix Houphouët-Boigny of Côte d'Ivoire) was studied by using the technique of opposite cultures, described by Hmouni et al. (1996). This test was carried out on the PDA medium. The fungal isolates and the phytopathogen were cultured on PDA medium and incubated for 7 days before the implementation of the antagonism test. After the 7 days of incubation, two explants including that of the pathogen and the antagonist agent were placed equidistant (3.5 cm) from the center of the Petri dish. The control consists of a subculture of the

pathogen in the Petri dish. These dishes were incubated in a culture oven at 27 °C. The percentage of inhibition was calculated using Eq. (1):

$$I (\%) = (1 - C_n/C_o) \times 100 \quad (1)$$

where:

C_n is the average diameter of the growth of the mycelium in the presence of the antagonist agent.

C_o is the average diameter of the growth of the mycelium in the absence of the antagonist agent.

2.3. Morphological identification of isolated fungal isolates

Fungal isolates from soils under vegetable cultivation have been described using the fungal determination key from Barnett and Hunter (1972). More recent ones by Pitt and Hocking (1997); Houbraken et al. (2014) were used to define the genres to which they belong.

2.4. Molecular identification of antagonist fungi

2.4.1. DNA extraction

The extraction of genomic DNA from the fungi was carried out according to the method described by White et al. (1990) with some modifications. Briefly, the liquid cultures of the fungi carried out previously with Potato Dextrose Broth (PDB) medium were centrifuged at 10,000 g for 3 min. After removing the supernatant, the pellet was homogenized in 400 µL of TBS buffer (0.4 M NaCl, 10 mM Tris-HCl pH 8 and 2 mM EDTA pH 8) then vortexed for 1 min. Then 40 µL of 20% SDS and 8 µL of Proteinase K (20 mg/mL) were added, then the mixture was incubated at 60 °C for 3 h. After incubation, 300 µL of 6 M NaCl were introduced into the various tubes which were also vortexed for 30 s and then centrifuged at 10,000 g for 30 min at 4 °C. The supernatants were transferred to tubes of 2 mL to which was added an equal volume of 100% Isopropanol. These tubes were incubated at -20 °C for 1 h and then centrifuged at 10,000 g for 20 min at 4 °C. The pellet was washed with 70% ethanol and then centrifuged again for 10 min at maximum speed. The pellet from the centrifugation was subsequently recovered and dried at 37 °C for 5 min. The DNA thus obtained was re-suspended in 300 µL of TE buffer (Tris-EDTA) sterile and stored at -20 °C to be amplified. The quantity and quality of DNA were estimated visually under UV rays after migration on 1% agarose gel against the 100 bp molecular weight marker DNA Ladder (Solis biodyne).

2.4.2. PCR amplification and sequencing

Fungal isolates were identified by sequencing the ITS (Internal Transcribed Spacer) regions of rDNA ITS1 (5'-GGA GTA AAA GTC GT A ACA GG-3') and ITS4 (5'-TCC TCC GCT TATTGA TATGC-3'). 4 µL of 5x Master Mix buffer (Solis Biodyne FIREPOL), 0.75 µL of each fungal primer at 10 µM, 5 µL of fungal DNA were combined in a final volume of 25 µL. Amplification consisted of an initial denaturation step of 2 min at 94 °C followed by 30 cycles of 30 s at 94 °C (denaturation), 30 s at 52 °C (hybridization) and 1 min at 72 °C (elongation) with a final elongation step of 10 min at 72 °C. Analysis of the PCR products was carried out by electrophoresis on 1% Agarose gel in 1X TBE buffer stained with SYBR SAFE (1%). The PCR products obtained were packaged and sent to Eurofin Genomics for Sanger sequencing according to the service provider's recommendations. Quality of the FASTA sequences was assessed and only good query sequences were used for the identification of fungi using the Megablast Algorithm of NCBI's Nucleotide BLAST Tool. The Fungi RefSeq ITS databases (Fungi FTP: <ftp://ftp.ncbi.nlm.nih.gov/refseq/TargetedLoci/Fungi/>) was targeted. It contains curated and re-annotated records of sequences from the ITS region in the nuclear ribosomal cistron, sourced from INSD records.

2.5. Production and extractions of secondary metabolites

The extraction of the secondary metabolites was carried out by first carrying out fermentation of the antagonist on a rice medium (40 g of rice

Table 1. *S. rolfssii* growth inhibition

Isolate	Description	Inhibition rate (%)
TmF 13	<i>Talaromyces calidicanius</i>	60,59
TmN 1	<i>Trichoderma yunnanense</i>	56,47
M1-12	<i>Trichoderma ghanense</i>	42,94
IIBio 5	<i>Trichoderma reesei</i>	40,59
M4-7	<i>Penicillium citrinum</i>	37,06
M4-8	<i>Penicillium citrinum</i>	35,29
IX1-1	<i>Clonostachys swieteniae</i>	31,18
IIX 1-2	<i>Talaromyces pratensis</i>	28,24
IIX2-13	<i>Talaromyces purpureogenus</i>	27,65
M2-10	<i>Talaromyces assiutensis</i>	27,06

for 80 mL of distilled water). To do this, a suspension of spores from a 7-day-old culture was produced in sterilized distilled water, then inoculated onto the previously sterilized rice medium. The rice media thus inoculated were incubated at 28 °C for 21 days. At the end of the incubation, the cultures were harvested in 1 L Erlenmeyer flasks. Then the extraction of the metabolites was carried out by adding 500 mL of ethyl acetate to each Erlenmeyer flask. The solution obtained after maceration was filtered three times through gas then through cotton wool. The collected filtrate was concentrated using a rotary evaporator to give the crude fungal extract.

2.6. Bioguided purification of bioactive secondary metabolites

The crude extracts were fractionated by chromatography on a silica gel column using an elution gradient of Cyclohexane/Ethyl Acetate (10/0/0–10). Fractions were pooled from their profile on TLC (Silica 60 F 254 Merck, Germany). The fractions obtained were evaluated against *S. rolfssii*. The fractions for were further purified in search of active molecules with the elution gradient indicated above. The 1D and 2D NMR spectra were recorded in DMSO- d_6 and Acetone- d_6 with Bruker 400 spectrometer using TMS as internal standard. The chemical shifts were given in δ (ppm). HR-ESI-MS spectra were obtained with an Applied Biosystem QSTAR Pulsar I mass spectrometer.

2.7. Evaluation of the biological activities of the fractions and of the purified compounds

For each fraction and purified compounds, a range of concentrations was achieved by the double dilution method, ranging from 5 to 20 mg/mL. Each concentration was spread beforehand on the PDA medium, then

the different dishes were inoculated with a fungal disc of *S. rolfssii* 5 mm in diameter (obtained from a culture of 7 days of incubation on solid PDA medium). The control was obtained from a disc of *S. rolfssii* alone on PDA. The petri dishes were incubated at 28 °C for 4 days. The percentage of growth inhibition I (%) is expressed by the reduction in the diameter of the fungal colony relative to the control, calculated using Eq. (2):

$$I (\%) = [1 - (D_{\text{test}} / D_{\text{control}})] \times 100 \quad (2)$$

with:

D_{test} : diameter of the colony in the test in mm.

D_{control} : control diameter of control colony in mm.

2.8. Statistical analysis

The means of the measurements of the diameters of inhibition carried out on the different days of collections were compared by ANOVA one way (GraphPad Prism 8.4.3).

3. Results

3.1. Antifungal activity of soil fungi against *S. rolfssii*

A total of 153 fungal isolates were collected, of which 10 (ie 15.3%) showed inhibitory activity against *S. rolfssii*. The antifungal activity was confirmed by measuring the diameter of inhibition of mycelial growth of *S. rolfssii*. The percent inhibition varied from 27.06 to 60.59% (Table 1). Alone isolates IIX2-13 and M2-10 exerted inhibition by diffusion of antifungal compounds into the agar with an observable zone of inhibition remained constant up to 11 days after incubation (Figure 1 and Figure 2). These antagonists were therefore selected for the identification of the compounds responsible for the antifungal activity.

3.2. Morphological identification of selected antagonist fungi

Microscopic identification of the antagonist fungi revealed the genera *Penicillium* (5 isolates) and *Trichoderma* (3 isolates).

3.3. Molecular identification of selected antagonist fungi

The request sequence, a DNA fragment corresponding to the different antagonists, was used to perform a BLASTn with the Megablast program. The data bank indicated was RNA types trains/ITS_RefSeq_Fungi. Table 2 lists the species identified by molecular biology. Analysis of the sequencing data revealed that the isolates fall into 4 genera with 4 species (Isolates TmF13, IIX 1–2, IIX2-13 and M2-10) for the genus

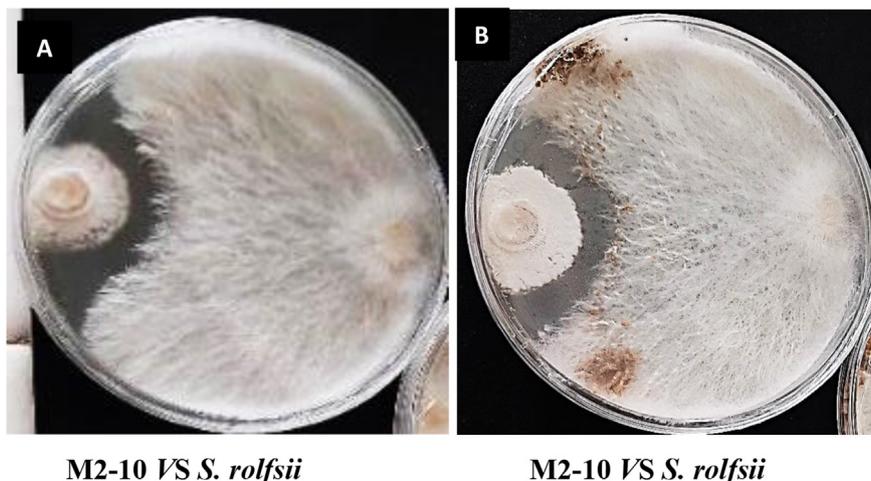


Figure 1. Dual culture test A: Isolate M2-10 and *S. rolfssii* after 5 days of incubation B: Isolate M2-10 and *S. rolfssii* after 11 days of incubation.

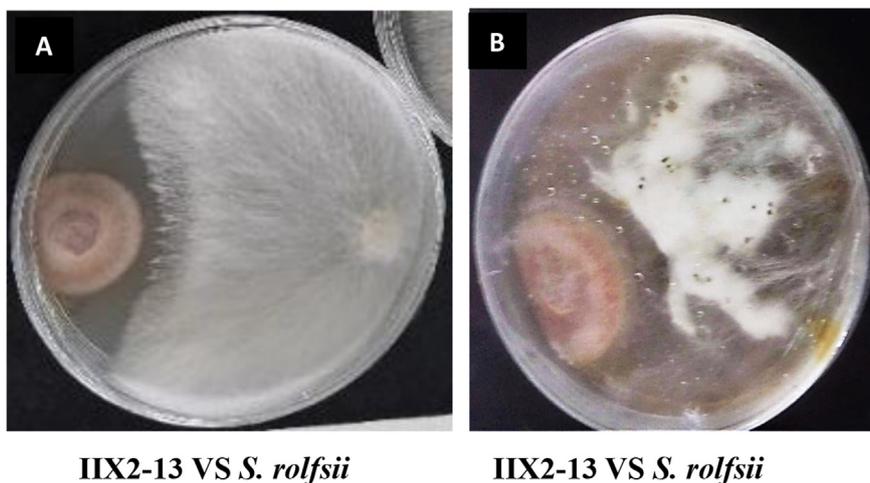


Figure 2. Dual culture test A: Isolate IIX2-13 and *S. rolfsii* after 5 days of incubation B: Isolate IIX2-13 and *S. rolfsii* after 11 days of incubation.

Talaromyces; 3 species for the genus *Trichoderma* (Isolates TmN 1, M1-12, and IIBio 5); one species for the genus *Penicillium* (Isolates M4-7, and M4-8); a species for the genus *Clonostachys* (Isolate IX1-1).

3.4. Biological activities of secondary metabolites

After extracting the cultures of the fungi, a crude extract of dark red color with a mass of 4 g was obtained for *T. purpureogenus* and a crude extract of brown color with a mass of 510 mg was obtained for *T. assiutensis*. Bioguided purification of crude extracts of *T. purpureogenus* led to compounds **1** (153 mg) and **2** (284 mg). The crude extract of *T. assiutensis* yielded compound **3** (168 mg) (Table 3).

Compound **1** with a concentration of 10 mg/mL completely inhibited the growth of *S. rolfsii*. On the other hand, compound **2** with the same concentration (10 mg/mL) exerted an inhibition of 70%. Compound **3** inhibited the mycelial growth of *S. rolfsii* by 38% (Table 4).

3.5. Purified antifungal compounds

Analysis of the mass spectrum of compound **1** made it possible to demonstrate a peak of molecular ion HR-ESI-MS m/z $[M + H]^+$ 383.111 (calc. for $C_{21}H_{18}O_7 + H^+$: 383.112), and m/z $[M + Na]^+$ 405.094 (calc. for $C_{21}H_{18}O_7 + Na^+$: 405.115). This analysis combined with the 1D and 2D NMR data made it possible to determine the chemical structure of **1** in accordance with the data in the literature corresponding to Mitorubrin (Figure 3). Analysis of the mass spectrum of compound **2** made it possible to demonstrate an HR-ESI-MS m/z $[M + H]^+$ 399.1074 (calc. for $C_{21}H_{18}O_8 + H^+$: 399.10711) and m/z $[2M + H]^+$ 797.21 (calc. for $C_{42}H_{36}O_{16} + H^+$). This analysis allowed to determine the chemical structure of **2** in agreement with the data of the literature,

Table 2. BLASTn results of 18S rRNA sequences of antagonist fungi.

Isolate	Description	% Identity	Accession nb
TmF 13	<i>Talaromyces calidicanus</i>	97,79	NR_103665.2
TmN 1	<i>Trichoderma yunnanense</i>	100	NR_134419.1
M1-12	<i>Trichoderma ghanense</i>	99,33	NR_120299.2
IIBio 5	<i>Trichoderma reesei</i>	99,83	NR_120297
M4-7	<i>Penicillium citrinum</i>	100	NR_121224
M4-8	<i>Penicillium citrinum</i>	99,43	NR_121224
IX1-1	<i>Clonostachys swieteniae</i>	96,89	NR_171105
IIX 1-2	<i>Talaromyces pratensis</i>	98,78	NR_165529
IIX2-13	<i>Talaromyces purpureogenus</i>	98,99	NR_121529
M2-10	<i>Talaromyces assiutensis</i>	96,74	NR_172040

which is the hydroxylated form of **1**, which corresponds to Mitorubrinol (Figure 3).

Analysis of the mass spectrum of compound **3** made it possible to demonstrate a high resolution molecular ion peak ESI $[2M + H]^+$ m/z 657.3849; $[M + H]^+$ m/z 329.1962 (calc. for $C_{17}H_{28}O_6 + H^+$: 329.1986). This analysis, combined with the 1D and 2D NMR data, made it possible to determine the chemical structure of the compound in accordance with the data in the literature. It was Spiculisporic acid (Figure 4).

4. Discussion

The tomato (*Solanum lycopersicum*) is subject to many fungal constraints in relation to the infectious potential of the soils. The city of Songon, located in the south of Côte d'Ivoire is a market gardening area, particularly tomatoes. This area is also described in the literature as being favorable to the proliferation of *S. rolfsii* (Bolou et al., 2015), thus devastating the tomato crops. However, the growth of *S. rolfsii* could be inhibited by other microorganisms that coexist in this same habitat through different naturally developed mechanisms of action. With a view to implementing biological control, soil investigations in this area were carried out. These investigations led to the isolation of 153 rhizospheric fungi.

The screening carried out from the 153 soil-based fungal isolates by means of a direct confrontation test on the PDA medium made it possible to select 10 fungi which inhibit the growth of *S. rolfsii*. The percentages of inhibition ranged from 27.06 to 60.59%. These results are statistically identical (ANOVA test, $F = 0.8042$; $p = 0.3932$). The

Table 3. Growth inhibition rate of *S. rolfsii* by crude extracts.

Crude extracts	Concentration (mg/mL)	Inhibition rate
<i>T. purpureogenus</i> IIX2-13	50	81%
	25	65%
	12,5	55%
<i>T. assiutensis</i> M2-10	50	68%
	25	58%
	12,5	46%

Table 4. Growth inhibition rate of *S. rolfsii* by compounds.

Compounds	20 mg/mL	10 mg/mL	5 mg/mL
1	100 %	100 %	40 %
2	90 %	70 %	32 %
3	38 %	04 %	00 %

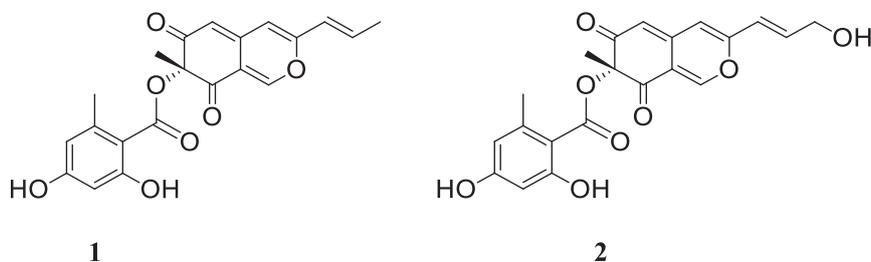


Figure 3. Structures of mitorubrin (1) and mitorubrinol (2).

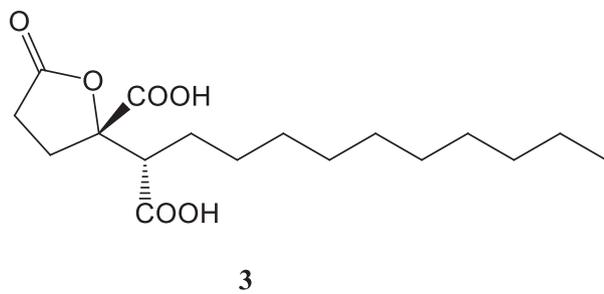


Figure 4. Structure of spiculisporic acid (3).

mechanisms of action observed during this inhibition were competition and antibiosis.

Rapid growth of some fungi (percent inhibition greater than 50%) inhibited mycelial growth of the pathogen. This phenomenon reminds us of the phenomenon of antagonism by competition of space and nutrients. This is because the depletion of nutrients caused by the rapid growth of the antagonist will slow down or inhibit the growth of the pathogen. Competition is particularly intense in the soil, which is an oligotrophic medium where the microorganisms are essentially at rest in the form of preservation. The rhizo-deposits released by the root system (supply of organic matter), will allow the activation of microorganisms in the rhizosphere of plants. Only the most competitive microorganisms will be able to take advantage of this nutrient supply and attempt to colonize the roots of plants (Aouar et al., 2019).

The second mechanism observed was antibiosis. This is based on the release by a microorganism of soluble or volatile secondary metabolites responsible for inhibiting the growth of a second microorganism. This inhibitory activity was observed by the appearance of a zone of inhibition followed by growth arrest of *S. rolfsii*.

Microscopic identification of the antagonist fungi revealed the genera *Penicillium* and *Trichoderma*. However, analysis of the ITS sequences confirmed one *Penicillium* in all 5 isolates and assigned 3 isolates to the genus *Talaromyces* and 1 isolate to the genus *Clonostachys*. This difference in result could be explained by the fact that the genera *Talaromyces* and *Penicillium* are anamorphs and therefore difficult to differentiate microscopically (Benjamin, 1955). In fact, the microscopic observation of *Talaromyces* is identical to that of the subgenus *Penicillium biverticillium*, so the subgenus *Penicillium biverticillium* has been transferred to the genus *Talaromyces* (Yilmaz et al., 2014). It is also difficult to differentiate on microscopy the genus *Penicillium* from the genus *Clonostachys* because both present a monoverticulate Conidiophore.

All isolates belonging to the genus *Trichoderma* ($n = 3$) exerted inhibition on the growth of *S. rolfsii*. *Trichoderma* species are rapid, invasive, filamentous colonizers, exhibiting a symbiotic relationship with plants. They exhibit antagonistic behavior against several phytopathogens such as bacteria, nematodes and in particular fungi by inhibiting their growth either by direct interaction (hyper parasitism, competition for nutrients and space, and antibiosis) or indirectly by improving plant growth and vigor and improving stress tolerance (Zhang et al., 2017).

These different properties explain why approximately 90% of biological fungal control agents against pathogenic microorganisms to date belong to different strains of *Trichoderma* (Sood et al., 2020). In addition to the *Trichoderma* species, 40% ($n = 4$) of the antagonists identified belong to the genus *Talaromyces*. *Talaromyces* species are cosmopolitan filamentous fungi that play various roles in natural ecosystems and in biotechnology. Several species of *Talaromyces* isolated from soil have the capacity to exert antagonistic activities against phytopathogenic fungi and to produce secondary metabolites unique to this genus (Manoch et al., 2013). As a result, several species of this genus including *T. flavus* are used in the biological control of fungal phytopathogens such as *Verticillium dahliae* and *Rhizoctonia solani* (Kakvan et al., 2013).

T. purpureogenus and *T. assiutensis* exerted the antibiosis mechanism. The low inhibitory powers of IIX2-13 and M2-10 are related to their slow growth, compared to that of *S. rolfsii*. In confrontation, their slow growth was compensated by the biosynthesis and the diffusion of antifungal compounds completely preventing the growth of *S. rolfsii*. This inhibitory activity was observed by the appearance of a zone of inhibition followed by stopping the growth of *S. rolfsii*. The zone of inhibition could be explained by the presence of toxic substances secreted by the antagonist agent. Indeed, the presence and size of the zone of inhibition have been used as evidence of the production of antibiotics by strains of *Trichoderma* (Barari, 2016). Some authors including Abouzeid (2014) and Gnancadja et al. (2015) used the mechanism of antibiosis to select different isolates likely to be biocontrol agents.

Mitorubrin and mitorubrinol obtained by bioguided fractionation of the crude extract of *T. purpureogenus* belong to the azaphilone family: a set of fungal secondary metabolites with a pyrone-quinone structure containing a highly oxygenated bicyclic nucleus and a chiral nucleus (Venkatchalam et al., 2018). They group together compounds exhibiting a broad spectrum of biological activity such as antimicrobial, antiviral, antioxidant, anti-inflammatory, cytotoxic and nematocidal activities. A synergistic effect of these two compounds could be at the origin of the inhibitory activity of *T. purpureogenus* against *S. rolfsii*. Pakora et al. (2018) have indeed shown a synergistic effect of gliovirin and viridin isolated from *Trichoderma sp* against *Phytophthora sp.*, The agent of brown rot in cocoa pods.

Spiculisporic acid resulting from the purification of the crude extract produced by *T. assiutensis* is a bioactive γ -butenolide, a mycotoxin found in certain species of *Talaromyces* such as *T. trachyspermus* (Yaguchi et al., 1994) and *T. purpureogenus* (Yilmaz et al., 2012). Spiculisporic acid is used commercially as a biosurfactant (Ishigami et al., 2000). This is the first time that spiculisporic acid has been reported to have been isolated from *T. assiutensis*. Hamza et al. (2017) showed nematophagous activity of *T. assiutensis* against juvenile strains of *Meloidogyne javanica*. The soluble n-hexane and chloroform fractions of *T. assiutensis* cultured filtrates showed strong antimicrobial activity against root rot fungi (Farhat et al., 2021).

5. Conclusion

This study made it possible to highlight the diversity of antagonistic fungi originating from the rhizosphere of tomato crops. Molecular

identification made it possible to highlight the antagonist fungi *T. purpureogenus* and *T. assiutensis* among the fungi exerting the mechanism of antibiosis. Bio-guided purification and NMR identified mitorubrin, mitorubrinol and spiculisporic acid as molecules responsible for inhibiting *S. rolfisii*. These molecules, already known but used in biotechnology, this study revealed another application of them, in particular in agriculture. Isolated from the same biotope as *S. rolfisii*, *T. purpureogenus* and *T. assiutensis* represent favorable candidates for biological control against *S. rolfisii*.

Declarations

Author contribution statement

Anne-Edwige Coulibaly: Performed the experiments; Wrote the paper.

Gilles Alex Pakora: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Aristide Berenger Ako: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Georges Elisée Ler-N'Ogn Dadé Amari, Carine Aya N'Guessan, Abo Kouabenan, Daouda Kone & Joseph Allico Djaman: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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