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Departament d'Acció Climàtica, Alimentació i Agenda Rural

Chaetomium and *Chaetomium*-like fungi as biological control agents for grapevine diseases

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Final degree project

Biochemistry and Molecular Biology Degree

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The healing of the land and the purification of the human spirit is the same process.

Masanobu Fukuoka

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Abstract

Cryptogamic diseases are one of the biggest problems in viticulture. Chemical treatments used for controlling them are harmful to ecosystems and humans, so it is imperative to find sustainable alternatives. We hypothesize that in the autochthonal microbiome from vineyards exist microorganisms with biocontrol potential against these diseases. 60 *Chaetomium* and *Chaetomium*-like strains have been isolated and identified from soil samples. *C. globosum, C. cochliodes* and *A. cupreus* have been tested *in vitro* against *Diplodia seriata* and *Didymella glomerata*. *C. globosum* and *C. cochliodes* are especially detachable against *D. glomerata* with 62,67% of inhibition of the mycelial growth.

Key words: biological control; antagonism; secondary metabolites; antifungal; *Chaetomium.*

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Abbreviations

ITS: Internal Transcribed Spacer

Tub2: β-tubulin

Rpb2: second largest subunit of ribosomal polymerase II

GTD: Grape Trunk Disease

BCA: Biological Control Agent

PCA: Potato Carrot Agar

DRBC: Dichloran Rose-Bengal-Chloramphenicol Agar

OA: Oatmeal Agar

PDA: Potato Dextrose Agar

PDB: Potato Dextrose Broth

MH: Mueller Hinton Agar

FMR: *Facultat de Medicina de Reus*

PIRG: Percentage Inhibitory of Radial Growth

CWDE: Cell-Wall-Degrading Enzymes

Introduction and background

This work has the aim to explore about biologic alternatives to chemical fungicides used against cryptogamic diseases in viticulture. Nowadays, cryptogamic diseases affect vineyards causing devastating damages and significant economic losses. Current treatments and solutions have negative human and environmental consequences, so it is imperative to explore sustainable and ecological alternatives. In this sense, biologic control could be a good alternative, especially those fungal-based. This work is introduced with a generally overview of fungi, a brief description of cryptogamic diseases and its current treatment methods. Later, biological control is described, emphasizing the fungal role of *Chaetomiaceae* family as potential biocontrol agents to fight against cryptogamic diseases.

1. Kingdom of Fungi

1.1. Overview of fungi

Fungi are eucaryotic organisms that traditionally constituted kingdom of fungi. This kingdom is included into Eukarya taxa and is subdivided into 12 phylums and more than 200 orders. The vast majority of the known fungi are included into Dikarya, which comprehends Basidiomycota and Ascomycota phylums (Ballen et al. 2017; Li et al. 2021).

Strict fungi are eucaryotic organisms with a chitin cellular wall and its cellular membrane contains ergosterol. Its nutrition is heterotrophic and absorptive: they secrete exoenzymes and degrade the substrates into molecules that are introduced later in the cell to obtain energy. This characteristic provides them a strategic position into carbon and nitrogen cycles in nature, as well as a great capacity to colonize too diverse substrates, making fungi ubiquitous. Fungi can be found practically in all types of environments and ecosystems, both terrestrial and aquatic (Cepero de García et al. 2012).

Fungal organisms can be unicellular, like yeasts, or pluricellular. Pluricellular fungi form tubular filaments named hyphae and are called filamentous fungi. The aggregate of hyphae is called mycelium. Dimorphic fungi can acquire both phenotypes, uni- and pluricellular, and usually are human and animal pathogens (Sil et al. 2015).

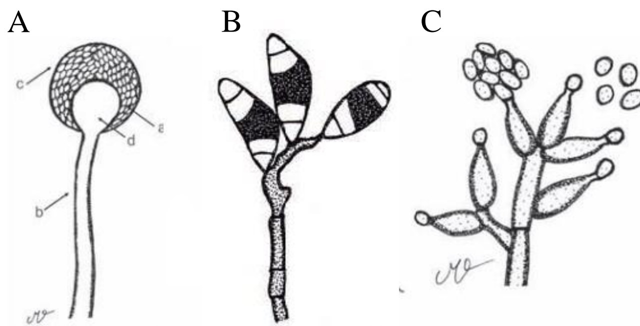

Figure 1. **Schematic representation of asexual reproduction structures of filamentous fungi**. **A**. Sporangium with sporangiospores. a) Sporangiospores. b) Sporangiophore. c) Sporangium. d) Columella. **B, C**. Conidia on conidiogenous cells. Adapted from Cepero de Garcia et al. 2012.

During their life cycle, fungi can propagate sexually or asexually. Some species can reproduce by both strategies, while others have only one type of reproduction. Sexual phase is known as teleomorph and asexual phase as anamorph. The organism in all its phases and forms is the holomorph.

Asexually, fungi can reproduce by fission, gemmation or sporulation. Fission and gemmation are typical of yeasts, while sporulation is characteristically of filamentous fungi. Sporulation consists in spore formation by mitosis, and spores are called mitospores. Mitospores can be sporangiospores or conidia. Sporangiospores are typical of a group of fungi traditionally known as phylum Zygomycota (nowadays reorganized into other phylums) (**Figure 1 A**), and conidia are characteristically from Dikarya (basidiomycetes and ascomycetes) (**Figures 1 B-C**). Asexual reproduction is the responsible of the massive formation of propagules genetically identical to the progenitor and allows the environmental colonization by fungi.

Sexually reproduction comprises genetic recombination and the origination of new genotypes, allowing the adaptation of individuals to different environments. Sexually reproduction is regulated by environmental, nutritional and genetical factors and usually is carried out in unfavorable external conditions. The process results in sexual haploid spore formation (**Figure 2**). Sexual spores can be basidiospores (from basidiomycetes) or ascospores (from ascomycetes). Usually, sexual spores have thick and pigmented walls, and can act as resistance forms of the fungi (Cepero de García et al. 2012).

1.2. Molecular fungal identification

Traditionally, fungi have been identified and classified by its phenotypic and physiologic characteristics. In many cases, especially ascomycetes, sexual and asexual phases from the same organism have been described independently and designated by different scientific names. The development of molecular biology has led to molecular

identification of organisms, and thereby to the reclassification of fungi according to DNA sequences and phylogenetic relationships. Hence, many holomorphs previously described as two different individuals, have been characterized as a unique one (Fajarningsih 2016; Cepero de García et al. 2012).

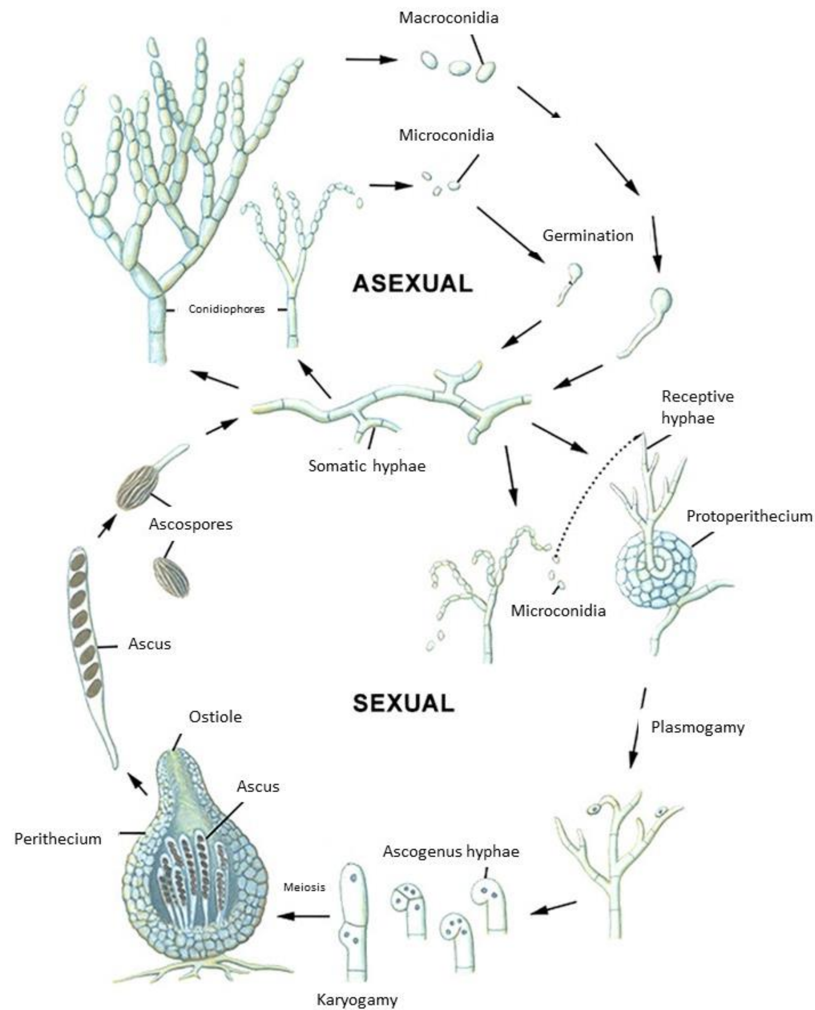

Figure 2. **Ascomycete's life cycle**. Sexual and asexual phases of reproduction. Adapted from Lowy, et al. 1991.

The mostly used method to molecular identification of fungi is "DNA barcoding". A "barcode" is a standardized, variable, and short -usually 700 base pairs approximately- DNA region, which is used as unique pattern to identify organisms. Ideally, these regions should be the same for all kingdoms, constant within a species, unique for each species, and variable enough to allow to contradistinguish between different species, also in those ones too evolutionary closely (Fajarningsih 2016).

The nuclear rRNA cistron is formed by 18S gene, encoding to nuclear ribosomal small subunit (SSU), 28S gene, encoding to nuclear ribosomal large subunit (LSU), 5.8S gene, and two internal spacers. This cistron is transcribed as a unit by RNA polymerase I, so internal spacers are called Internal Transcribed Spacers, ITS1 and ITS2, respectively (**Figure 3 A**). LSU and SSU have been studied to fungal identification but have been demonstrated being too conserved sequences and do not allow to identify at species level. ITS region is a highly polymorphic non-coding sequence able to separate sequences into species level, exists in numerous copies in the genome and is easy to amplify, hence, has been proposed as the primary fungal barcoding for fungi. However, it does not offer sufficient information for some fungal genera, and in those cases is necessary to include the amplification of secondary barcodes (Schoch et al. 2012; Fajarningsih 2016). Some of these secondary DNA regions are protein-coding genes, like β-tubulin (*tub2*), translation elongation factor 1-a (*tef1a*) or the subunit of ribosomal polymerases. In fact, second largest subunit of ribosomal polymerase II (*rpb2*) has been proposed as the alternative molecular marker for fungal identification for many species, as it allows to distinguish between some species better than ITS (Větrovský et al. 2016) (**Figure 3 B**).

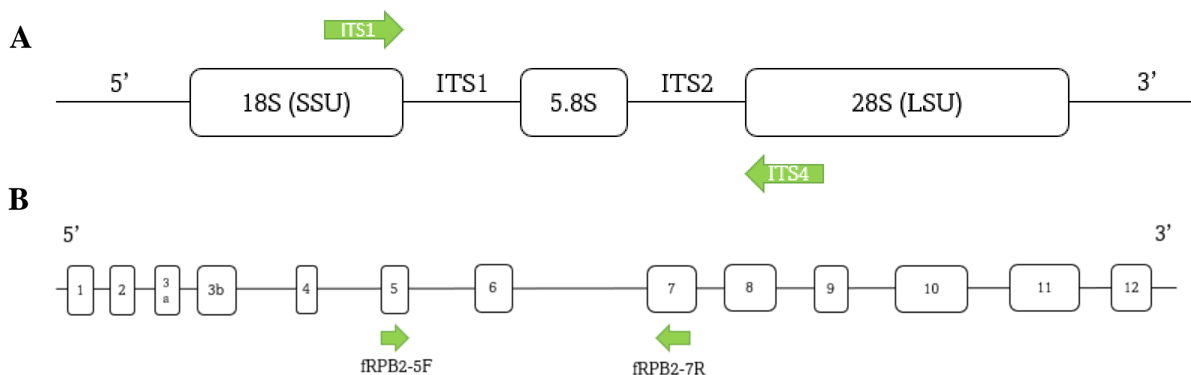

Figure 3. Representation of barcodes used for fungal molecular identification. **A**. Nuclear rRNA cistron (modified from Fajarningsih 2016). **B**. RPB2 gene (modified from Liu et al. 1999). Green arrows represent the position of specific primers commonly used to PCR amplification of these DNA regions (ITS1 and ITS4, RPB2-5F and RPB2-7R, respectively).

1.3. Cryptogamic diseases in agriculture

Fungi have been identified to cause infections in plants, which are known as cryptogamic diseases. These plant diseases can be caused by authentic fungi (Kingdom Fungi) or pseudofungi known as oomycetes. Both types of organisms share the capacity to produce spores, remaining in soil during decades in latent state. These spores activate themselves when temperature and humidity conditions turn favorable and can cause primary

infections on plants. These diseases propagate with extreme facility, causing enormous damages to the crops and economical struggle to the farmers. An example of this damage was the affectation in 2020 of the Catalan vineyards with *Plasmopara viticola,* an oomycete commonly known as mildew (**Figure 4**), which caused until a 70% of losses to agriculturists, especially those in ecological regime (Diari Oficial de la Generalitat de Catalunya, Núm. 8229 - 21.9.2020). In the other hand, fungal diseases include a large range of affectations, which can affect any part of the plant, like grey mold disease, caused by *Botrytis cinerea* (Williamson et al. 2007) which affects to grapes and leaves, or Grape Trunk Diseases (GTDs) that affects the wood. GTDs are considered the major problem of vineyards and the most widespread, important, and destructive fungal diseases in grapevines. To this day, their complete eradication is still not possible (de Almeida et al. 2020). Some of the causal agents of GTDs are *Seimatosporium vitis, Phaeoacremonium minimum, Diplodia seriata* and *Didymella glomerata* (Díaz et al. 2013; Cobos et al. 2022; de Almeida et al. 2020; Mondello et al. 2018; Cimmino et al. 2021)

Figure 4. Symptoms observed for grapevine downy mildew caused by *Plasmopara viticola* on rachis. Adapted from Zhang et al. 2017.

1.3.1. Use of pesticides in the treatment of cryptogamic diseases

Historically, cryptogamic diseases in agriculture were treated with natural products, directly obtained from animal, vegetal or mineral sources (Tudi et al. 2021). From XVII century to 1940's decade, fungicide treatments consisted in inorganic synthetic materials, mainly based on copper or sulfur compounds. Most of these products had to be applied repeatedly, at high rates (10 to 20 kg/ha) and did not always give good control, also sometimes were phytotoxic. At middles of XX century, modern fungicides were introduced, like mancozeb or chlorothalonil, the most widely used. Most of these

fungicides were used at lower rates (1,5 to 3 kg/ha) and were less phytotoxic while more active than the inorganic ones. However, mostly of these products resulted highly toxic and pathogens developed resistances (Conway 2020; Tudi et al. 2021).

The excessive use of chemical pesticides in agriculture is risky to the human and environmental health. These chemicals can enter the environment and persist on it after being applied. Once in the soil, pesticides can undergo processes such as transfer (moving from the target site to other environments) and degradation, which produces new chemicals that can be more toxic or pollutant than the original, altogether, causing soil, environment, and water pollution. This pollution can expose general population to pesticides residues trough water, air, or food (Bernardes et al. 2015). Additionally, fungicides can affect organisms other than target pathogens, including animals, insects, and plants (Tudi et al. 2021), but also other microorganisms and especially non-pathogen fungal species that are beneficial to the crops, causing biodiversity loss (Duke 2018; Keiblinger et al. 2018). Many fungi are flexible in their ability to undergo genetic recombination, hybridization, or horizontal gene transfer. Fungicides application reduces quantity and diversity of mycological microbiome and apply a biological pressure that led to microorganisms evolving and developing resistances to the chemicals, resulting in the final persistence of highly virulent phytopathogens (Fisher et al. 2012). Some of these pathogens also have crossed interspecies barriers, causing infections both in humans and plants, being an important human health threat (Silva et al. 2019; Gauthier et al. 2013). Finally, climate change factors, as temperature increasing or rainfall pattern changes, results in higher pesticides needing (Tudi et al. 2021). All these reasons have triggered political and social concern about the use of pesticides in agriculture. Because of that, European Union has driven the creation of the 'European Green Deal', a document which reflects the political and social intentions to mitigate global warming and environmental issues and collect different objectives to design a fair, healthy and environmentally-friendly food system, such as reducing significantly the use and risk of chemical pesticides and promoting ecologic agriculture (European Comission COM2019 640 final).

However, ecological agriculture still uses chemical pesticides like Bordeaux mixture, based on copper sulfate and lime arsenic (Tudi et al. 2021). Although preventive fungicides based on copper are theoretically non-pollutant because its active principle naturally exists in ecosystems (Pérez et al. 2007), its continual use as broad spectrum

foliar fungicide can cause bioaccumulation of copper on soil, pollution, and affectations to microbiome equilibrium, leading to changes in recycling nutrients and soil fertility (Komárek et al. 2010; Wightwick et al. 2008). Hence, it is necessary to find sustainable alternatives to copper products that can be efficient against plant's disease agents and respectful with the beneficial microbiome of the crops. In this sense, European Commission aims to promote the introduction of bioproducts for crop protection (European Comission COM2020 381 final). Some of these types of products already in use are *Bacillus thuringiensis* against lepidoptery insects or *Trichoderma*'s based fungicides.

1.3.2. Biological Control Agents as an alternative to chemical products

A promising alternative to substitute chemical products in agriculture are Biological Control Agents (BCAs). Biological control, or biocontrol, is the reduction of density or activity of a pathogen by one or more microorganisms, ocurring naturally or by introduced microorganisms. These microorganisms are named BCAs or antagonists, and usually are searched and isolated from soil, endosphere or phyllosphere of the plants to compete with and/or destroy plant pathogens (Thambugala et al. 2020; Arya et al. 2010). While chemical fungicides only have temporary effects and need frequent applications, BCAs are capable of stablish itself in the ecosystem, reproduce and colonize the phyllosphere and/or the rhizosphere (Silva et al. 2019). This make them especially interesting to being studied as a strategy compatible with the new model of agriculture.

The antagonists can exhibit several direct or indirect mechanisms of action involved in biological disease control (**Figure 5**). Direct mechanisms include antibiosis (the antagonist produce an inhibitory metabolite or antibiotic), mycoparasitism (the antagonist derives nutrients from a fungal host) and secretion of extracellular enzymes. Indirect mechanisms involve induced resistance (the antagonist has the ability to induce defense response of the plant against pathogens), growth enhancement (the antagonist promote plant growth, sometimes by secretion of hormones such as gibberellic acid, while reduce the effects of the disease) competition for space and/or nutrients and detoxification of virulence factors (Thambugala et al. 2020).

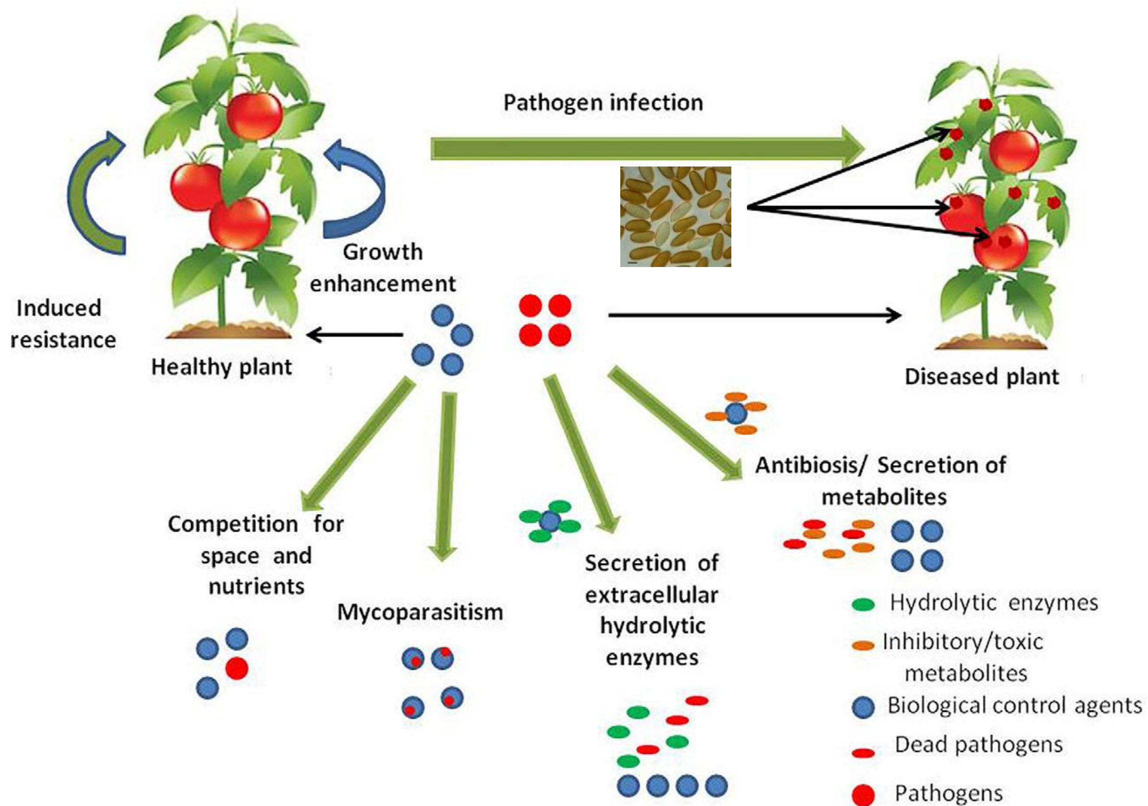

Figure 5. **Mechanisms of antagonism involved in biological control of plant fungal diseases by fungi.** Adapted from Thambugala et al. 2020.

Biological control has been used for more than two hundred years. In 1874 was introduced the term antagonism, and since then, several experiments have been done to application into agriculture. The potential for the application of fungal biological control agents against plant pathogens has largely increased because fungi have a higher reproductive rate (sexually and asexually), a short generation time, are target specific and can survive in absence of the host (Thambugala et al. 2020). Interesting fungal genera for biologic control are *Trichoderma, Alternaria, Penicillium, Epicoccum, Talaromyces* and *Chaetomium* (Arya et al. 2010).

1.4.*Chaetomiaceae* family and *Chaetomium* as BCAs

Chaetomium species belong to the family *Chaetomiaceae,* order Sordariales, class Sordariomycetes and division of Ascomycota (Schoch et al. 2020). *Chaetomium* species have been reorganized taxonomically several times by phylogenetic analysis of different DNA sequences, especially ITS and partial β-tubulin gene sequences. Nowadays, *Chaetomiaceae* family is divided into 50 genera: *Chaetomium sensu stricto* and 49 more genera, where we can find *Collariella, Botryotrichum, Arcopilus, Arxotrichum, Dicotomophilus, Humicola* and *Farrowia,* among others (Schoch et al. 2020). These

genera are morphologically similar to *Chaetomium* species; however, phylogenetical relations have shown that are different and distant species and are commonly known as *Chaetomium*-like species.

Morphologically, the genus *Chaetomium* is commonly recognized by having ostiolate ascomata with a perithecial wall covered by hairs, producing asci and ascospores (**Figure 6**) (Wang et al. 2016).

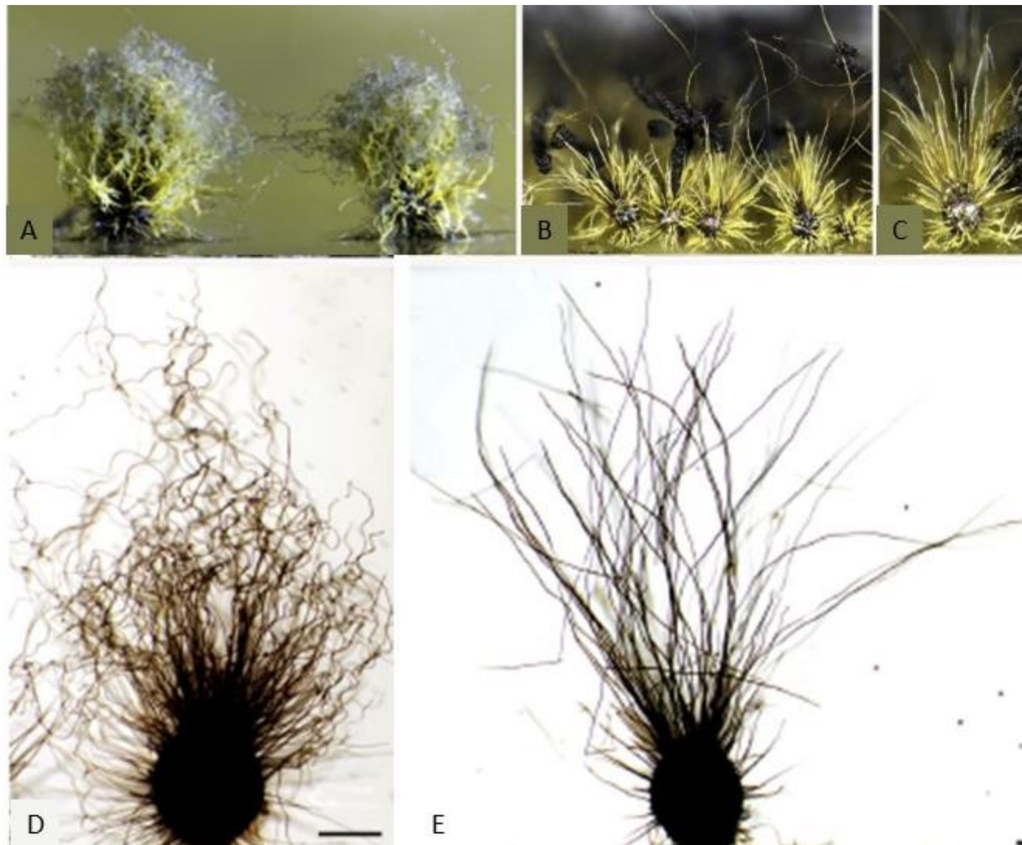

Figure 6. ***Chaetomium globosum.*** **A**, **B**, **C**: Mature ascomata on Oatmeal Agar (OA), side view. **D**, **E**: Ascomata mounted in lactic acid. **A**, **D**: DTO 319-B2 strain. **B**, **C**, **E**: CBS 666.82 strain.

Chaetomium is a ubiquitous genus, whose species are usually isolated both from indoor and outdoor habitats. Outdoors, are common in soil, decaying wood, and plants, and indoors are usually found on substrates like paper or textiles, as they can degrade cellulose. They also are well-known for their ability to produce a large variety of bioactive metabolites, where we can find chaetoglobosins, epipolythiodioxopiperazines, azaphilones, depsidones, xanthones, anthraquinones, chromones, terpenoids, and steroids (Zhang et al. 2012). These bioactive compounds are interesting for many disciplines like medicine, pharmaceutical industry, biotechnology, and agriculture. For example, many anticancer compounds have been isolated from endophytic *Chaetomium globosum*

(Kumar et al. 2021), and some antimicrobial and cytotoxic metabolites have been isolated from an endophytic *Chaetomium brasiliense* (Promgool et al. 2021).

In agriculture, antimicrobial and antifungal compounds are of especially interest, as they can be useful in biologic control of phytopathogens. *Chaetomium* species have been tested with *in vitro* experiments against diverse phytopathogens as *Diplodia seriata, Neofusicoccum parvum,* both associated to grape trunk diseases (Silva-Valderrama et al. 2021), and *Phytophthora nicotianae,* causal agents of disease in large variety of plants, including horticultural crops (Hung et al. 2015).

Chaetomium globosum is the most common species of the genre and the most reported as BCA. It has demonstrated efficacy in double culture assays against horticultural pathogens like *Sclerotium rolfsii, Macrphomina phaseolina, Sclerotinia sclerotium* and *Fusarium oxysporum* (Kumar et al. 2021; Huu et al. 2016), and *Botrytis cinerea,* the causative agent of grey mold disease in grapevine (Garoé et al. 2012), among others. Many interesting metabolites from *C. globosum* have been described. For example, chaetoglobosins A, C, D, E, G and R (**Figure 7 A-F**) have shown antifungal activity against *Coniella diplodiella,* the causative agent of grapevine white rot (Zhang et al. 2013); chaetoviridin A (**Figure 7 G**) has shown antifungal activity against *Magnaporthe grisea,* a phytopathogen from rice; and chaetoglobosin B (**Figure 7 H**) has been reported active against *Mycbacterium tuberculosis,* the causative agent of tuberculosis in humans (Ni et al. 2008). Antibiotic metabolites have been purified from *C. globosum* too, like chaetoglocins A and B (**Figure 7 I-J**), pyranones with activity against Gram-positive bacteria (Ge et al. 2011).

Chaetomium cochliodes and *Chaetomium cupreum* (now taxonomically classified as *Arcopilus cupreus*), have been reported as BCAs too. In this sense, a bioproduct based on 22 strains of these two species known as Ketomium® was commercialized in Thailand (Thailand Patent No. 6266, International Code: AO 1N 25/12). This product has demonstrated activity against many phytopathogens like *Fusarium oxysporum* or *Phytophthora palmivora,* among others (Soytong et al. 2001). At his turn, *Arcopilus cupreus* is a source of many agricultural interesting metabolites, like the azaphilone rubrorotiorin (**Figure 7 K**) with antifungal activity values comparable to amphotericin (Kanokmedhakul et al. 2006).

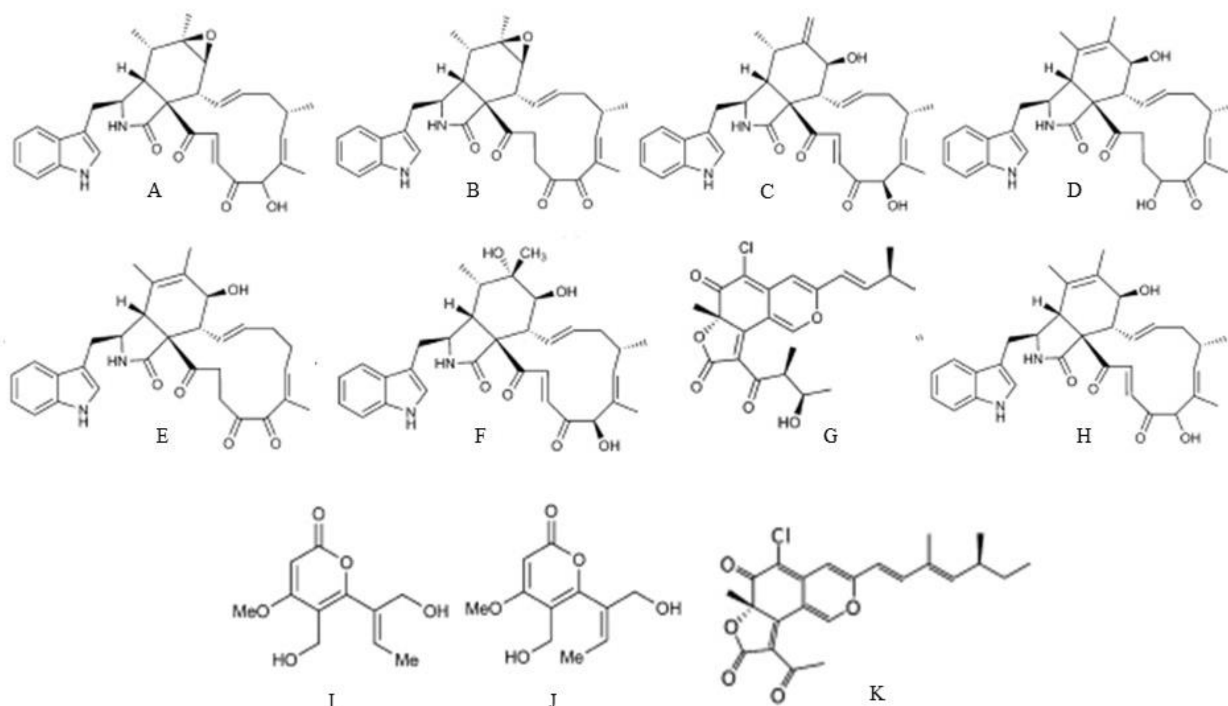

Figure 7. **Interesting metabolites from *Chaetomium* strains**. Modified from Fatima et al. 2016 and Kanokmedhakul et al. 2006.

Finally, some *Chaetomium* strains have demonstrated to be growth stimulators of plants too: in cucumber, *C. globosum* can associate symbiotically with roots and promote plant metabolism and hormone production, and also produces phytohormones like indole-3-acetic acid, gibberellin and zeatin by itself (Tian et al. 2022), and in *Salvia miltiorrhiza* has shown capacity of accumulation of bioactive constituents like tanshinones and salvianolic acids (Zhai et al. 2018).

In this sense, *Chaetomium* and *Chaetomium*-like species are of extreme interest as potential biocontrol agents for agriculture. This genus has been widely studied against food crop phytopathogens, but poorly considered against grapevine pathogens, so it is an interesting field to develop with the aim of reduce chemical pesticides and fertilizers used in viticulture.

Hypothesis and objectives

We hypothesize that in the autochthonal microbiome of the crops exist microorganisms with interesting activities for agriculture application and antagonistic potential against phytopathogens that affects those crops and that *Chaetomium* and *Chaetomium*-like species from *Chaetomiaceae* family can accomplish these requirements. Thereby, the aim

of this work is to isolate and identify these fungi with biocontrol potential against fungal pathogens of *Vitis vinifera* from soil and rhizosphere of the vineyards and demonstrate their antagonistic activity *in vitro*.

Specific objectives:

- Isolate *Chaetomiaceae* species from local crops and identify genus and specie of each strain by specific barcodes (*tub2*).
- Prove the antagonist activity from the isolates against fungal crop pathogens by dual culture to assay their antifungal activity.
- Culture them in liquid medium to generate secondary metabolites and assay the filtrate against different strains of bacteria to study their antibiotic activity.

Methods and materials

1. Sample processing

1.1. Sampling

Soil samples were collected between May and November 2021 from different crop fields of collaboration companies "Clos Mogador SL" (Camí Manyetes s/n, 43737, Gratallops, Tarragona) and "Verd Camp Fruits SAT" ('Masia Barrabeig', Camí de Mont-roig s/n, 43850 Cambrils, Tarragona). Plastic bags were used for collecting samples from the soil and the rhizosphere from diverse points of the vineyards and horticultural crops.

1.2. Culture media

Potato Carrot Agar (PCA) is a poor medium without added sugars which simulates natural environment of fungi. Dicloran Rose-Bengal-Chloramphenicol agar (DRBC) is a selective medium for fungi. Oatmeal Agar (OA) is a poor medium, specially selected to stimulate fungal macrospore formation. Potato Dextrose Agar or Broth (PDA and PDB) are rich media that allow mycelium development of nearly all fungi. Mueller Hinton (MH) is the standard medium used for antimicrobial susceptibility tests.

1.3. Fungal culture

Soil samples were homogenated manually into the same collection bags. To isolate fungal species, each sample was cultured as follows: 1 g of soil was diluted into 10 ml of sterile distilled water, and this was distributed into 4 Petri dishes. Half of the dishes were mixed with Dicloran Rose-Bengal-Chloramphenicol agar (DRBC; 2.5 g peptone, 5 g glucose, 0.5 g $KH2PO4$, 0.25 g $MgSO4$, 12.5 mg Rose Bengal, 100 mg chloramphenicol, 1 mg

dicloran, 10 g agar, 500 mL distilled water) melted at 45 °C; and the other half with Potato Carrot Agar (PCA; 10 g potato, 10 g carrot, 100 mg chloramphenicol, 6.5 g agar, 500 mL distilled water), also melted at 45 ºC. All mediums were supplemented with chloramphenicol, an antibiotic used to avoid bacterial contamination, and 100 µL of dieldrin, an insecticide to avoid acarus contamination. Once solidified, from every sample, one PCA plate and one DRBC plate was incubated at 25 ºC, and the other two plates were incubated at 15 ºC. All plates were examined weekly with a stereomicroscope for up to 4–5 weeks.

1.4. Spores' activation

Independently, same soil samples underwent a spore activation procedure with phenol. This process is used to activate latent spores by stress, and it is used to isolate fungi that do not develop with the classic methods of culture. In this case, 1 g of soil was diluted into 5 mL of phenol 2%, mixed, and incubated at room temperature for 5 minutes. After incubation, phenol was discarded by decantation, and the sample was diluted into 10 mL of sterile distilled water. From that moment on, samples were processed as described on fungal culture (3.3).

1.5. Fungal isolation

To achieve pure cultures, ascomata or ascospores of presumable *Chaetomiaceae* colonies of all samples were transferred with a sterile dissection needle to plates with Oatmeal agar supplemented with chloramphenicol (OA; 15 g oatmeal, 100 mg chloramphenicol, 6.5 g agar, 500 mL distilled water) plates and incubated at 25 °C in darkness.

1.6. Fungal conservation

Each fungus obtained in pure culture was transferred to PCA and OA agar inclined tubes, to stablish a work bank. To further fungal conservation, all isolates were deposited into the fungal collection of the Faculty of Medicine, Reus (FMR), by lyophilization and preservation in water.

Lyophilization consist in introduce mycelium from a 14-days grown strain in PDA in glass vials with skimmed milk 10%, which acts as cryoprotectant agent, and summit it to the lyophilization protocol of the freeze dryer. Preservation in water consist in the introduction of agar plugs of fungal colonies from a 14-days grown strains in PDA into glass vials of water (both distilled water and normal water). Both types of vials were sealed and conserved at room temperature.

2. Fungal identification

2.1. Phenotypic identification

Preliminary morphological identification was done by macroscopical and microscopical observation. To macroscopic identification, fungal colonies were observed in PDA and OA agar at 7 and 14 days of incubation at 25 ºC in darkness. To microscopical observation, some perithecium or ascomata were transferred to a drop of Shears solution (3 g potassium acetate, 150 mL distilled water, 60 mL glycerin, 90 mL ethanol 95%) deposited on a slide.

2.2. DNA extraction, amplification, and electrophoresis

Isolates were cultured on PDA for 7–14 days at 25 °C in darkness. The DNA was extracted through commercial "FastDNA" kit (MP Biomedicals, Thermo Fischer Scientific Inc., Massachussets, USA). DNA was diluted with milli-Q water to a final concentration of 30 ng/µl. For molecular identification of the isolates, specific molecular markers (barcodes) were amplificated by PCR. PCRs were carried out with EmeraldAmp GT PCR Master Mix kit (Takara Bio Inc., Shiga, Japan) and specific primers in **Table 1**. EmeraldAmp GT PCR Master Mix is a 2X premix composed of a DNA polymerase, optimized reaction buffer, dNTPs, and a density reagent. This simplifies PCR assembly; it is only necessary to add primers, template, and water (also proportioned in the kit), at concentrations shown in **Table 2**. PCR conditions are shown in **Table 3**. After PCR, the reaction mixture can be applied directly to a gel for analysis, as the premix also contains a green dye that separates into blue and yellow dye fronts when run on an agarose gel. Electrophoresis were performed in manually prepared 1% agarose gels (1 g agarose in 100 mL of TBX 1X and 3 µl of RedSafe (iNtRON Biotechnology Inc., Korea)) and were carried out during 45 minutes at 90 V. Gel images were obtained with a transilluminator and ImageLab software (Bio-Rad, California, USA). Those samples with a unique band were correctly amplificated (**Figure 1s**, Suplementary material).

Table 1. Sequences of the primers from the molecular *barcodes* to molecular identification of the strains.

Locus	Primer	Direction	Sequence	Reference
ITS	ITS5	Forward	GGAAGTAAAAGTCGTAACAAGG	(White et al. 1990)
	ITS4	Reverse	TCCTCCGCTTATTGATATGC	
tub2	Bt2a	Forward	GGTAACCAAATCGGTGCTGCTTTC	(Glass et al. 1995)
	Bt2b	Reverse	ACCCTCAGTGTAGTGACCCTTGGC	
rpb2	RPB2-5F	Forward	GA(T/C)GA(T/C)(A/C)G(A/T)GATCA(T/C)TT(T/C)GG	(Liu et al. 1999)
	RPB2-7Rc	Reverse	CCCAT(A/G)GCTTG(T/C)TTRCCCAT	

Table 2. Final concentration and volume of Master Mix for PCR amplification.

Component	Final concentration	Volume for one reaction
EmeraldAmp GT ®	1X	12,5 µl
dH_2O	-	9,5 µl
Primer Forward	0,2 µM	0,5 µl
Primer Reverse	0,2 µM	0,5 µl
Template (30 ng/µl)	< 10 ng/µl	2 µl
		Final volume: 25 µl

Table 3. PCR conditions to amplify molecular *barcodes* used to identify the isolates.

Locus	Initial denaturation	Nº of cycles	Denaturation	Hybridization	Elongation	Final elongation
ITS, *tub*2	94 ºC, 5’	35	94 ºC, 30”	56 ºC, 45”	72 ºC, 1’	72ºC, 7’
***rpb*2**	94 ºC, 5’	35	95 ºC, 45”	56 ºC, 1’	72 ºC, 1’45”	72ºC, 7’

2.3. Sequencing and molecular identification

For DNA sequencing, DNA concentration from amplificated sample was measured (**Figure 2s**, Suplementary material), and content was diluted with milli-Q water to a final concentration of 20 ng/µl for each 100 base pairs of the barcode sequence (for example, if *rpb*2 barcode is 900 pb long, the sample was diluted to a final DNA concentration of 180 ng/µl). Amplified products were sequenced at Macrogen (Madrid, Spain) for the same primers used in amplification. Sequences obtained were analyzed with BioEdit 7.2 software (BioEdit LTD, Manchester, UK) to select correct sequenced fragments from the results. Identification was obtained using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) by comparation of obtained sequences with sequences deposited into GenBank from NCBI. Those sequences with an identity percentage higher than 98% were considered correctly identified at species level, while those with an identity percentage lower than 98% require further phylogenetic study to determine if are novel species.

3. Antagonistic activity of fungal cultures

3.1. Dual culture assay

Three isolated and identified strains were selected to the antagonistic capacity assay: *Chaetomium globosum* (Ch11, FMR 19699), *Chaetomium cochliodes* (Ch12, FMR 19700) and *Arcopilus cupreus* (Ch28, FMR 19716) (Soytong et al. 2021). Two grapevine

phytopathogens were obtained from FMR collection, granted by Dr. Alberto M. Sthigel: *Diplodia seriata* (FMR 18743) and *Didymella glomerata* (FMR 18750), to assay antagonistic activity from selected strains.

Antagonism capacity of the strains was evaluated by two different methods of dual culture assay. Each strain, including the pathogens, was cultured in PDA during 7 days at 25 ºC. In both methods, a 5 mm plug of this culture, both pathogen and the antagonist, was placed on the opposite side of a PDA plate (Stracquadanio et al. 2020). In the second method, the PDA plate was separated into two zones by a 1 cm column without agar. The first method evaluates the inhibition growth for direct contact and production of nonvolatile substances, whereas the second method determines the possibility of inhibition due to volatile substances, because the colonies are not in direct contact. All plates were incubated at 25 ºC and radial growth was measured at days 5, 7 and 14. Plates with a single agar disc of the pathogen were used as controls. Three repetitions of each assay were made.

The percentage inhibitory of radial growth (PIRG) was calculated using the formula from Stracquadiano et al. (2020).

$$PIRG\% = \frac{(Dc - Dt)}{Dc} \cdot 100$$

Where PIRG is percent of growth inhibition; Dc is growth rate of the pathogen (control) and Dt is growth rate of the pathogen in presence of *Chaetomium*.

3.2. Liquid culture and metabolite production

To obtain secondary metabolites produced by selected strains of *Chaetomium*, one 7 mm plug of agar obtained from actively growing margin of a 7-day PDA culture, was used to inoculate a 500 mL flask containing 250 mL of sterile potato dextrose broth (PDB). Liquid cultures were incubated for 10 days at 30 ºC under stirring (100 rpm), as described by Stracquadiano et al. (2020). The cultures were filtered into a sterile Falcon flask using a 10 mL syringe with 0,45 µm filter coupled. For each filtered suspension, control plates were inoculated to ensure sterility: one plate of MH agar and another one of PDA agar were inoculated with 100 µl of filtrate and spred with a Drigalsky spatula. MH plate was incubated at 37 ºC for 24 hours while PDA plate was incubated at 25 ºC for 7 days.

3.3. Agar diffusion test

The antibiotic capacity of the metabolites obtained of the three *Chaetomium* strains was tested in agar diffusion tests with different strains of bacteria. Selected bacteria were part of the collection bank of Facultat de Medicina de Reus and are showed in **Table 4**. One part of the assayed bacteria were *Aeromonas* strains obtained of the soil or vegetables. Another part were clinical isolated bacteria of infected wounds. Bacteria were defrosted from collection tubes at -80 ºC, cultured during 24 h on Mueller Hinton (MH; 2 g beef extract, 17.5 g acid hydrolysate of casein, 1.5 g starch, 17 g agar, 1 L distilled water) agar plates and revised to make sure that were pure cultures.

Table 4. Bacterial strains used in antibiotic agar diffusion test.

Strain code	Origin	Identification
T41-7	Soil	*Aeromonas media*
Ale-15D	Lettuce	*Aeromonas caviae*
Ble-15C	Lettuce	*Aeromonas caviae*
-	Wound's smear	*Enterococcus faecalis*
-	Wound's smear	*Klebsiella oxytoca*
-	Wound's smear	*Escherichia coli*
-	Wound's smear	*Serratia marcescens*
-	Wound's smear	*Aeromonas sp.*

To agar diffusion test, first, a bacterial suspension was made using a sterile inoculating loop to transfer some bacterial colonies to sterile distilled water in a tube, and turbidity was adjusted at 2 MacFarland scale ($BaSO_4$ 9,60 · 10^{-5} M) (bioMérieux SA, Marcy l'Etoile, France). A swab was immersed in the suspension and the entire surface of the agar of a MH plate was rubbed to achieve a complete sowed surface. After that, 5 mm plugs of agar were removed and 20 µl of *Chaetomium* culture's filtrate was placed in the holes. PDB without inoculation was used as control in every plate. Plates were incubated at 37 ºC for 24 hours and diameter of the inhibition halos was measured. As a qualitative assay, no inhibition was calculated. MH control plates were incubated at 37 ºC for 24 hours, while PDA control plates were incubated at 25 ºC for 7 days.

Results

1. Molecular identification

60 *Chaetomium* and *Chaetomium*-like strains have been isolated in pure culture from a total of 17 soil samples. From these, 48 isolates have been identified with the *tub*2

sequencing (**Table 5**), and 12 are still unidentified (complete table is shown at (Suplementary material, **Table 1s**). Identified strains include *Chaetomium, Arcopilus, Collariella, Arxotrichum, Dicotomophilus, Amesia* and *Botyotrichum* genres. All *Chatomiaceae* strains have been isolated from phenol activated samples.

12 *Chaetomium* strains have been isolated from vineyards soil samples, including 6 different strains of *C. carinthiacum,* 2 of *C. globosum* (one of them, corresponding to FMR 19709, needs a further phylogenetic analysis to establish if corresponds to *C. globosum* or it is a new species), one *C. cochliodes,* one *C. atrobrunneum,* one *C. indicum* and one *C. subfunicola. C. atrobrunneum* and *C. cochliodes* have been isolated from plant cover samples, both in DRBC plates cultured at 25 ºC. *C. globosum* corresponding to code Ch11 FMR 19699, three strains of *C. carinthiacum* (Ch3 FMR 19691, Ch10 FMR 19698 and Ch30 FMR 19718) have been also isolated from plant cover samples cultured at 25 ºC. *C. globosum* grew on DRBC plates, while *C. carinthiacum* both in PCA and DRBC plates. *C. indicum, C. subfunicola* and 3 strains of *C. carinthiacum* (Ch2 FMR 19690, Ch32 FMR 19720, Ch56 FMR 19894) have been isolated from grapevine rhizosphere samples. *C. subfunicola, C. indicum* and *C. carinthiacum* (Ch2 and Ch32) grew on PCA plates incubated at 25 ºC, while *C. carinthiacum* Ch56 grew on DRBC at 15 ºC.

From *Chaetomium*-like strains, 8 isolates correspond to *Arcopilus cupreus.* 6 of them have been isolated from grapevine rhizosphere, and 2 from vineyard's plant cover. Growth conditions were PCA or DRBC at 25 ºC, with exception of Ch54 FMR 19892 which grew on PCA at 15 ºC. No other species from *Arcopilus* have been isolated. 7 strains correspond to *Collariella* species. From these, only 3 strains were isolated from

Table 5. Identified *Chaetomiaceae* species of the study, with sample, isolation conditions, BLAST and GenBank information. In blue there are indicated those strains correctly identified, in orange those that need further analysis to identify them with security and in black those that could be new species (<98% of identity in BLAST's results).

Code	FMR	ID	BLAST % identity	Sequence length	GenBank accession number	Sample origin	Isolation conditions
Ch1	19689	***Arcopilus cupreus*** strain CBS 560.80	100%	396 pb	KX976926.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Garnatxa. "Solà" vineyard	Ph-PCA 25ºC
Ch2	19690	***Chaetomium carinthiacum*** strain C87	100%	387 pb	HM365299.1	"	"
Ch3	19691	***Chaetomium carinthiacum*** strain C87	100%	238 pb	HM365299.1	**Clos Mogador**. Central plant cover, Garnatxa. "Solà" vineyard	"
Ch4	19692	***Arxotrichum succineum*** *strain CBS 119769*	98,55%	421 pb	MK919400.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Garnatxa Negra. "L'hort" vineyard	"
Ch5	19693	***Chaetomium atrobrunneum*** *C64*	99,27%	421 pb	HM365294.1	**Clos Mogador**. Central plant cover (10 years), Garnatxa Negra. "L'hort" vineyard	Ph-DRBC 25ºC
Ch6	19694	***Arxotrichum succineum*** *strain CBS 119769*	98,55%	421 pb	MK919400.1	**Clos Mogador**. Rhizosphere *Vitis vinífera*. Centennial vineyard	Ph-PCA 25ºC
Ch7	19695	***Amesia atrobrunnea*** *strain CBS 250.75*	98,82%	437 pb	KX976917.1	"	"
Ch8	19696	***Arxotrichum succineum*** *strain CBS 119769*	98,73%	395 pb	MK919400.1	"	"
Ch9	19697	***Dicotomopilus subfunicola*** CBS 794.83	100%	168 pb	KX977013.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Syrah. "Obaga" vineyard	"
Ch10	19698	***Chaetomium carinthiacum*** strain C87	99,37%	159 pb	HM365299.1	"	Ph-DRBC 25ºC
Ch11*	19699	***Chaetomium globosum*** strain CBS 160.62	99,45%	181 pb	KT214742.1	"	Ph-PCA 25ºC
Ch12*	19700	***Chaetomium cochliodes*** strain CBS 155.52	99,75%	395 pb	JN256147.1	"	Ph-DRBC 25ºC
Ch13	19701	***Collariella bostrychodes*** strain CBS 163.73	98,60%	508 pb	KX976983.1	"	"

Ch14	19702	***Chaetomium funicola*** strain CBS 158.52	100%	321 pb	JF772461.1	**VerdCamp Fruits**. Rhizosphere *Quercus ilex*	"
Ch15	19703	***Chaetomium coarctatum*** strain CGMCC 3.14299	100%	251 pb	JN256194.1	"	"
Ch17	19705	***Chaetomium funicola*** *CBS 158.52*	98,16%	432 pb	JF772461.1	"	"
Ch18	19706	***Chaetomium funicola*** *CBS 158.52*	100%	402 pb	JF772461.1	"	"
Ch19	19707	***Collariella bostrychodes*** strain CBS 163.73	100%	121 pb	KX976983.1	"	"
Ch21	19709	***Chaetomium globosum*** strain LC4145	96,27%	403 pb	KP336886.1	"	"
Ch22	19710	***Collariella bostrychodes*** strain DTO 319-C4	97,65%	385 pb	KX976985.1	**VerdCamp Fruits**. Rhizosphere *Prunus persica*	"
Ch24	19712	***Collariella bostrychodes*** strain CBS 163.73	100%	367 pb	KX976983.1	**VerdCamp Fruits**. *Citrullus lanatus* crops	"
Ch26	19714	***Chaetomium biapiculatum*** *strain LC3853*	99,50%	403 pb	KP336857.1	"	"
Ch27	19715	***Arcopilus cupreus*** strain CBS 560.80	98,46%	389 pb	KX976926.1	**Clos Mogador**. Central plant cover, Garnatxa. "Solà" vinyeard	Ph-DRBC 25ºC
Ch28*	19716	***Arcopilus cupreus*** strain CBS 560.80	97,90%	441 pb	KX976926.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Garnatxa. "Solà" vinyeard	"
Ch29	19717	***Arxotrichum succineum*** strain CBS 119769	99,25%	408 pb	MK919400.1	"	"
Ch30	19718	***Chaetomium carinthiacum*** strain C87	100%	387 pb	HM365299.1	**Clos Mogador**. Central plant cover. "Obaga" vinyeard	"
Ch31	19719	***Arcopilus cupreus*** strain CBS 560.80	98,40 %	375 pb	KX976926.1	"	Ph-DRBC 25ºC
Ch32	19720	***Chaetomium carinthiacum*** strain C87	99.75%	397 pb	HM365299.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Syrah. "Obaga" vinyeard	Ph-PCA 25ºC
Ch33	19721	***Chaetomium subfunicola*** strain C37	99,74%	392 pb	HM365300.1	"	"
Ch34	19722	***Arcopilus cupreus*** strain CBS 560.80	98,52%	406 pb	KX976926.1	**Clos Mogador**. Rhizosphere *Vitis vinífera*. Centennial vinyeard	"

Ch35	19723	***Chaetomium indicum*** strain C2	99,75 %	396 pb	HM365301.1	"	"
Ch37	19725	***Arxotrichum succineum*** strain CBS 119769	99,21%	378 pb	MK919400.1	"	
Ch45	19733	***Collariella carteri*** CBS 128.85	100%	395 pb	KX976989.1	**Clos Mogador**. Central plant cover (10 years), Garnatxa Negra. "L'hort" vineyard	"
Ch46	19734	***Collariella carteri*** CBS 128.85	100%	397 pb	KX976989.1	**VerdCamp Fruits**. Rhizosphere *Curcubita maxima. "*Salis"	Ph-PCA 15ºC
Ch48	19736	***Arcopilus cupreus*** *strain CBS 560.80*	98,40 %	375 pb	KX976926.1	**Clos Mogador**. Central plant cover. "Obaga" vineyard	Ph-DRBC 25ºC
Ch49	19737	***Dichotomopilus subfunicola*** *CBS 794.83*	99,47%	383 pb	KX977013.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Garnatxa Negra. "L'hort" vineyard	Ph-PCA 25ºC
Ch50	19888	***Chaetomium biapiculatum*** LC3852	99,23%	392 pb	KP336856.1	**VerdCamp Fruits.** Rhizosphere *Brassica oleracea* i *Foeniculum vulgare*	Ph-DRBC 15ºC
Ch52	19890	***Collariella bostrychodes*** DTO 319-C4	99,74%	388 pb	KX976985.1	"	Ph-PCA 15ºC
Ch54	19892	***Arcopilus cupreus*** strain CBS 560.80	98,77%	406 pb	KX976926.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Carinyena. "Bienvenido" vineyard	"
Ch55	19893	***Arcopilus cupreus*** strain CBS 560.80	98,06%	411 pb	KX976926.1	**Clos Mogador**. Rhizosfere Syrah and Garnatxa. "Obaga" vineyard	Ph-DRBC 25ºC
Ch56	19894	***Chaetomium carinthiacum*** strain C87	97,58%	206 pb	HM365299.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Carinyena. "Bienvenido" vineyard	Ph-DRBC 15ºC
Ch57	19895	***Chaetomium cuniculorum*** CBS 121.57	99,75%	410 pb	KP900709.1	**VerdCamp Fruits**. Rhizosphere *Curcubita maxima. "*Salis"	"
Ch58	19896	***Chaetomium undulatum*** C78	99,21%	381 pb	HM365279.1	"	Ph-PCA 25ºC
Ch59	19897	***Botyotrichum murorum*** CBS 173.68	100%	404 pb	KX976934.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Garnatxa. "Carretera" vineyard	Ph-PCA 25ºC
Ch60	19898	***Arxotrichum succineum*** strain CBS 119769	99,25%	402 pb	MK919400.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Garnatxa. "Carretera" vineyard	Ph-PCA 25ºC
Ch61	19899	***Arxotrichum succineum*** strain CBS 119769	96,30%	107 pb	MK919400.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Carinyena. "Bienvenido" vineyard	Ph-PCA 15ºC

vineyards samples, corresponding to *C. bostrychodes* (Ch13 FMR 19701, Ch19 FMR 19707) isolated from rhizosphere, and *C. carteri* (Ch45 FMR 19733) isolated from central plant cover, both species on DRBC plates at 25 ºC.

5 strains of *Arxotrhicum succineum* have been isolated from *V. vinifera*'s rhizosphere, all at 25 ºC from both PCA and DRBC plates. 2 *Dicotomophilus subfunicola* strains have been isolated from grapevine's rhizosphere, both at PCA 25 ºC. One strain of *Botyotrhicum murorum* and one of *Amesia atrobrunnea* have been isolated from grapevine's rizosphere in PCA at 25 ºC. No other species from *Arxotrichum, Dicotomophilus, Botyotrichum* or *Amesia* have been isolated.

2. Phenotypic identification

Three assayed *Chaetomiaceae* strains were also identified phenotypically. Colonies were examined in PDA and OA agar after 14 days of culture (**Figure 8 A-C**). At 14th day, ascomata were mounted on Shears solution to microscopic identification (**Figure 8 D-F**).

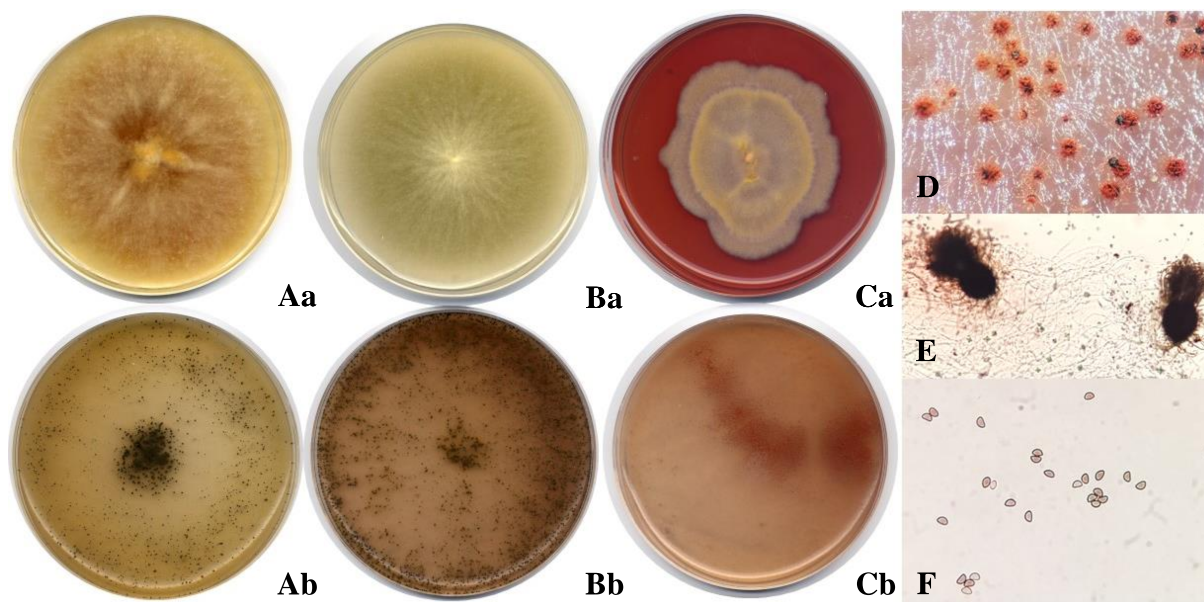

Figure 8. Morphological characteristics of *Chaetomium* strains. *Chaetomium globosum* (**A**), *Chaetomium cochliodes* (**B**), *Arcopilus cupreus* (**C**) 14 day-cultures in PDA (**a**) and OA (**b**) agar. **D**. *A. cupreus'* ascomata in OA. **E**. *A. cupreus'* ascomata mounted in Shears solution, 40x. **F**. Ascospores, 100x.

3. Antagonistic activity against fungal pathogens

In the first method, inhibition of the mycelial growth of *Didymella glomerata* and *Diplodia seriata* by direct contact and/or by secretion of metabolites of three *Chaetomiaceae*'s family strains was assessed. All assayed species showed an inhibition of the mycelial growth of *Didymella glomerata* (**Figure 10, 1**) while any of them showed

a strong inhibition of the *Diplodia seriata* colonies (**Figure 10, 2**). **Table 6** and **Figure 9** shows de percentage inhibition of radial growth (PIRG) observed on 5, 7 and 14 days after the culture. All *Chaetomium* strains that have been tested show more than a 40% of inhibition of the mycelial growth of *Didymella glomerata* on the 14th day, and *C. globosum* and *C. cochliodes* show the highest PIRG values, both with 62,67%. In some cases, no inhibition was observed on 5th day, when the colonies have not entered in contact. *A. cupreus* inhibits *D. glomerata*'s growth at day 14 without direct contact between the fungi, as a 0,5 cm halo can be appreciated (**Figure 10, 1-c**). In the case of *C. globosum* and *C. cochliodes,* colonies enter in contact as they grow (**Figure 10, 1-a, b**).

Table 6. Percentage inhibition of radial growth (PIRG) in dual culture assay. Results are expressed as the ± standard error of PIRG after 5-, 7- and 14-day incubation.

Strains	*C. globosum*			*C. cochliodes*			*A. cupreus*		
	5th day	**7th day**	**14th day**	**5th day**	**7th day**	**14th day**	**5th day**	**7th day**	**14th day**
Diplodia seriata	28,72 ± 1,78	38,22 ± 3,08	36,89 ± 5,39	23,59 ± 0,89	35,11 ± 0,77	36,67 ± 0,00	32,89 ± 3,36	32,89 ± 3,36	33,33 ± 0,00
Didymella glomerata	5,55 ± 1,92	34,11 ± 1,34	62,67 ± 0,00	0,00	30,23 ± 0,00	62,67 ± 0,00	0,00	12,40 ± 5,85	47,56 ± 1,54

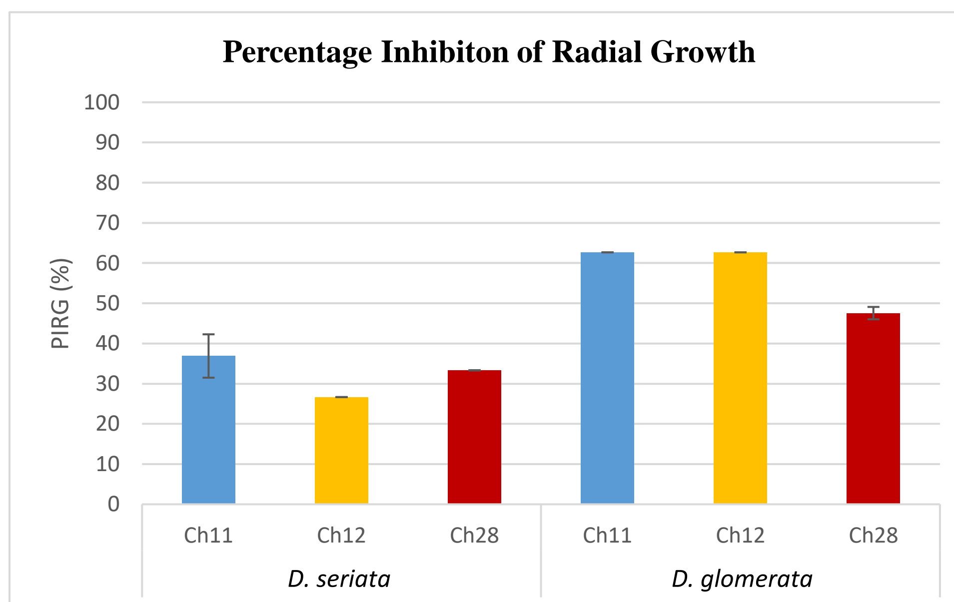

Figure 9. Percentage Inhibition of Radial Growth. Results of Table 6 at 14th day. Error bars correspond to standard error calculated.

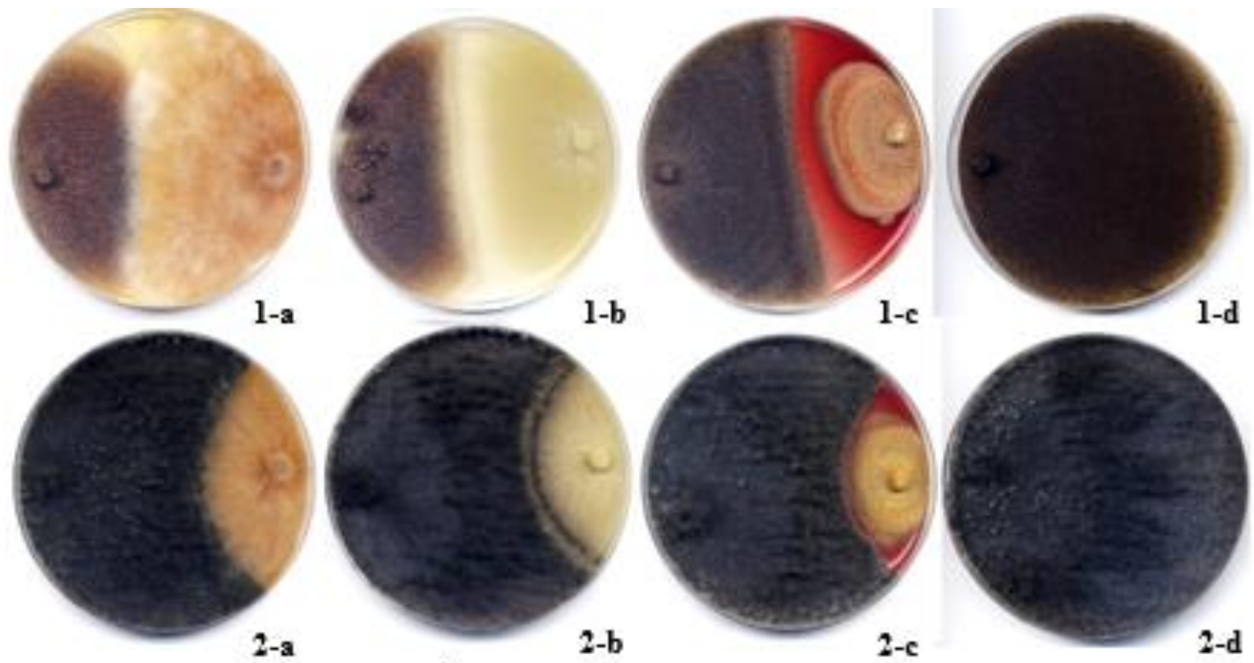

Figure 10. Dual culture assay after 14-days of incubation. *Didymella glomerata* (**1**) and *Diplodia seriata* (**2**) in coculture (left side of each plate) with *Chaetomium globosum* (**a**), *Chaetomium cochliodes* (**b**), *Arcopilus cupreus* (**c**) and control (**d**).

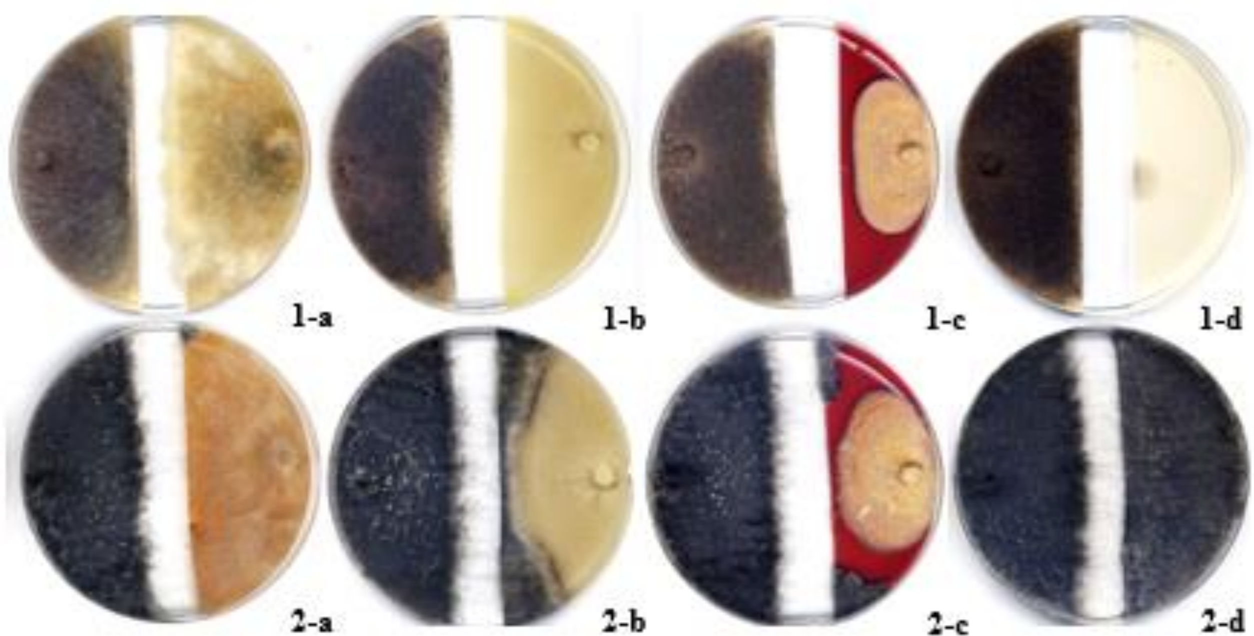

Figure 11. Dual culture assay with divided agar after 14-days of incubation. *Didymella glomerata* (**1**) and *Diplodia seriata* (**2**) in coculture (left side of each plate) with *Chaetomium globosum* (**a**), *Chaetomium cochliodes* (**b**), *Arcopilus cupreus* (**c**) and control (**d**).

The second method, the double culture test with divided agar, makes possible to evaluate the inhibition of the mycelial growth of pathogens by secretion of volatile substances. No inhibition was observed in this case (data not shown in table 3). None of the tested strains had an indirect inhibition to *Didymella glomerata* as showed in (**Figure 11, 1**). Results

for *Diplodia seriata* are not valid because *D. seriata*'s colony has been trespassed to the *Chaetomium*'s part of agar, so indirect mechanisms cannot be evaluated (**Figure 11, 2**).

4. Antibacterial activity on solid medium

The three strains tested in dual culture (*C. globosum, C. cochliodes* and *A. cupreus*) have been cultured in liquid medium to obtain its metabolites. This have been done also with *Arxotrichum succineum* (Ch6, FMR 19694); *Amesia atrobrunnea (*Ch7, FMR 19695); *Chaetomium biapiculatum* (Ch26, FMR 19714) and another strain of *Arcopilus cupreus* (Ch31, FMR 19719). Culture's filtrates have been tested against different bacteria to explore its antibiotic activity. None of the selected strains have shown antibiotic activity in agar diffusion test (data not shown), except for *C. globosum* (Ch11), which filtrate seems to slightly inhibit *Aeromonas* sp.'s growth (**Figure 12 A**, red arrow).

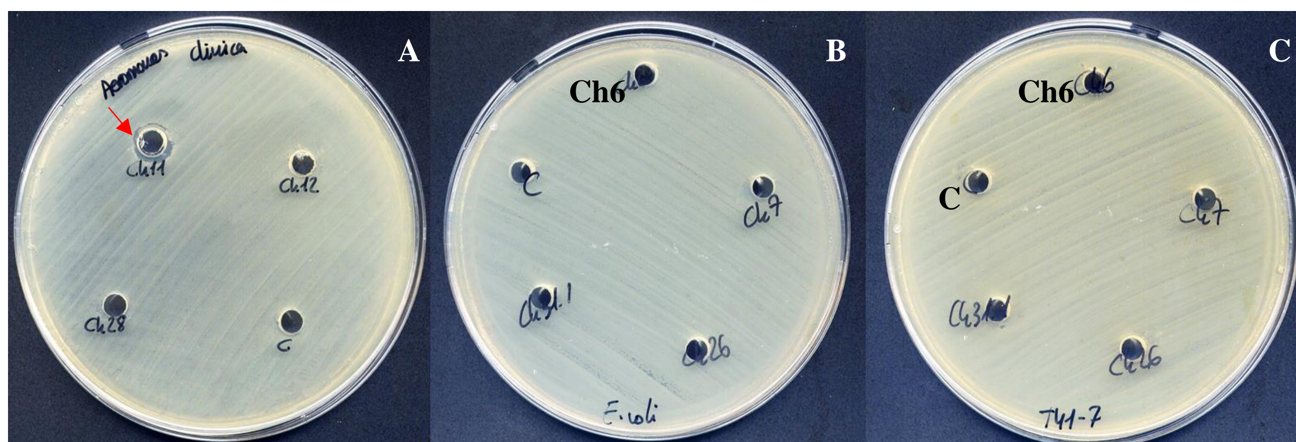

Figure 12. Agar diffusion test results. A. *Aeromonas* sp. clinical isolate **B.** *Escherichia coli* clinical isolate. **C.** *Aeromonas media* T41-7 isolated from soil. Ch11, *C. globosum* FMR 19699; Ch12, *C. cochliodes* FMR 19700 and Ch28, *A. cupreus* FMR 19716; Ch6, *Arxotrichum succineum* FMR 19694; Ch7, *Amesia atrobrunnea* FMR 19695; Ch26, *Chaetomium biapiculatum* FMR 19714; Ch31.1, *A. cupreus* FMR 19719; C, control (sterile Potato Dextrose Broth).

Discussion

1. Isolation and identification

31 strains have been isolated from vineyard soil's samples, including 12 different *Chaetomium* and 8 different *Chaetomium*-like species. The most common species identified has been *Arcopilus cupreus,* with a total of 8 isolates, while 6 strains correspond to *Chaetomium carinthiacum,* 5 to *Arxotrichum succineum* and 3 correspond to *Collariella* genre, one *Collariella carteri* and two *Collariella bostrychodes.* Two strains have been identified as *Chaetomium globosum,* other two as *Dicotomophilus subfunicola,*

and single isolates are identified as *Chaetomium cochliodes, Chaetomium atrobrunneum, Chaetomium indicum, Chaetomium subfunicola, Botyotrichum murorum* and *Amesia atrobrunnea.* All these species have been reported in outdoors environments (Abdel-Azeem 2020), but there is no information about Spanish vineyards' populations. Although biodiversity of *Chaetomium* and *Chaetomium*-like species is poorly reported, especially in soil, *Chaetomium globosum, C. cochliodes, C. indicum* and *C. atrobrunneum* have been isolated commonly from large variety of crop foods (such as rice, beans, soybeans, maize, tomato, etc.). *C. carinthiacum, C. succineum* (= *Arxotrichum succineum*) or *C. murorum* (= *Botyotrichum murorum*) are lesser common but also reported on vegetals such as rice or cereals (Abdel-Azeem 2020),. While in our study -although data is preliminary- *Arcopilus cupreus* and *Chaetomium carinthiacum* are the most reported species, in literature *C. globosum* seems to be the most common species indoors (Wang et al. 2016) and outdoors (Abdel-Azeem 2020), near followed by *C. cochliodes* species. As endophyte, *C. globosum* is the most reported and studied species, and has been isolated from a large variety of plants, including *Vitis vinifera* (Garoé et al. 2012). *Chaetomium cupreum* or *Arcopilus cupreus* has been reported as endophyte too (Haruma et al. 2018). The distribution along the species of our study do not match with the reported data. However, not all the strains have been identified from the total isolates and data are too preliminary and uninterpretable, so it is necessary to have the complete information to draw concluding information.

2. Antagonistic activity against fungal pathogens

Chaetomium sp. has been largely studied as biocontrol agent. Here, all *Chaetomium* strains assayed have shown more than a 40% on inhibition of the mycelial growth of *Didymella glomerata,* so in relation with our results, *C. globosum, C. cochliodes* and *A. cupreus* are good antagonists against *D. glomerata. Didymella glomerata* is also known as *Phoma glomerata* and, generally, has been reported as an endophyte, but recently has been associated with Grape Trunk Disease (Cimmino et al. 2021). In this sense, *Chaetomiaceae* species have not been tested against *Didymella glomerata,* and comparations cannot be done.

In case of *Diplodia seriata,* PIRG values are lower than in *D. glomerata*'s experiments for the three *Chaetomium* selected strains. *Chaetomium* sp. has been previously described as efficient against *D. seriata*, with a percentage of inhibition near the 60% (Silva-Valderrama et al. 2021). However, all our assayed strains can inhibit the pathogen

mycelial growth less than 40%. *C. globosum* and *C. cochliodes* reach more than a 36% of inhibition, while *A. cupreus* only arrives to a 33%. However, the most detachable difference between *C. globosum* and *C. cochliodes* with *A. cupreus* is the type of inhibition: *A. cupreus* can inhibit the growth of the phytopathogens without entering in contact with hyphae, as a halo without growth is created in direction to the pathogenic strain. Hence, inhibition caused by *A. cupreus* is not caused by direct contact between the fungi, but by the secondary metabolites secreted by the antagonist (**Figure 9, c**). Many antifungal metabolites have been reported of *A. cupreus:* for example, Jiang et al. (2017) identified an exo-1,3-β-glucanase from a biocontrol strain of *Chaetomium cupreum* (= *Arcopilus cupreus*). Many other proteins, enzymes, and molecules are isolated from *C. cupreum,* including proteases, chitinases, glucosaminidases and other glucanases, that contribute to antifungal activity (Daley et al. 2017). *A. cupreus* also secrete a red pigment, known as oosporein (6,60-tetrahydroxy-2,20 dimethyl-5,50-bi-p-benzoquinone) that have shown antifungal activity against *Rhizoctonia solani* (Kanokmedhakul et al. 2006). These secondary metabolites are part of the antibiosis or competition mechanisms of the fungus and are responsible of the antagonistic characteristics of *Arcopilus cupreus.* Instead, in the case of *C. globosum* and *C. cochliodes*, we can assume that antagonistic activity is mainly caused by direct mechanisms, as colonies enter in contact as they grow. Direct mechanisms can be antibiosis or mycoparasitism, both in which antagonist secrete enzymes that affects to the pathogen. *C. globosum* secrete chaetoviridins and chaetoglobosins, polyketides with antagonistic activity against numerous phytopathogens (Daley et al. 2017). *C. globosum* secrets also cell-wall-degrading enzymes (CWDE). Fungal cell walls are constituted mainly by chitin and glucan, so CWDE are mostly chitinases and glucanases. *C. globosum* have been reported as mycoparasitic of phytopathogens by secretion of chitinases which expression is triggered in presence of cell wall fragments from the pathogens (*Rizhoctonia solani, Sclerotinia slecrotiorum,* among others) (Liu et al. 2008). Regarding *C. cochliodes* as BCA, some metabolites have been identified (Wang et al. 2020), but mechanisms for biocontrolling are not clear. As the colonies enter directly in contact, it is probably that *C. cochliodes* act by mycoparasitism or by antibiosis using CWDE.

On the other hand, this study could not demonstrate the growth's inhibition of the phytopathogens by volatile metabolites, as the method of the divided agar on Petri dish was uninterpretable. We can conclude that dividing the agar is an invalid method and that

it is necessary to evaluate volatile metabolites on divided commercial Petri dishes, for avoid the colonization of the agar by the contrarian fungal strains. However, we can assume that antagonism from *Chaetomium* strains against *D. glomerata* and *D. seriata* is due to mycoparasitism, involving cell-wall-degrading enzymes, as well as by the secretion of antifungal metabolites to the media. In this sense, it was not expected that volatile metabolites caused inhibition to the pathogens.

3. Antibacterial activity

Respecting the antibiotic assay, none of the *Chaetomium* filtrates have shown antibacterial activity in agar diffusion assay. *C. globosum* showed a slightly inhibition of bacterial growth for *Aeromonas* sp., but only in one of the three repetition plates. It cannot be considered as a valid antibiotic activity due to the extremely thin of the halo and the lack of reproducibility. In this sense, it was expectable that *C. globosum, C. cochliodes* and *C. cupreum* revealed antibiotic activity against bacteria (Zhao et al. 2022; Wang et al. 2019; Zhang et al. 2007). Although antibacterial metabolites have been reported for these species, the experiments for them usually have been carried out from the purified metabolite. In this work we have tested the crude filtrate, with an unknown concentration and, obviously, with a vast mixture of metabolites on it; in addition, only 20 µl of the filtrate have been used, so it is possible that the antibiotic activity is present but not evident in the experiment's conditions. Few information is available from *Arxotrichum succineum, Amesia atrobrunnea* and *Chaetomium biapiculatum* or its antibacterial metabolites, so we cannot expect concrete results of its antibacterial activity.

Besides that, it is important to highlight that secondary metabolites' production is highly influenced by external conditions (Barrios-González 2018). In our case, we have cultured fungal strains in liquid medium and shaking conditions for 10 days. We can assume that these conditions are insufficient for maximal secondary metabolites production, and more quantity of these metabolites will be produced in more incubation time or other temperature or shaking conditions. For example, in their experiments for obtaining *C. globosum*'s metabolites, Zhao et al. (2017) culture the fungus in PDB for 12 days at 25 ºC and 150 rpm and Kumar and colleagues (2021) have cultured it 7 days in shaking followed by 14 days in BOD at 28 ºC and 65% RH. Hence, the best conditions to metabolite production should be studied.

Finally, we must consider that the most common antibacterial activity reported from *Chaetomiaceae*'s metabolites is against Gram-positive bacteria (Fatima et al. 2016), and we have tested mainly against Gram-negative strains.

4. *Chaetomiaceae* as BCAs

Chaetomium strains have great potential as biocontrol agents (Soytong et al. 2021) but some of the species have been reported as pathogens. *Chaetomium* are saprophytic ascomycetes and only cause human diseases occasionally and especially in immunocompromised patients, nevertheless, they can induce several mycoses including onychomycosis, sinusitis, pneumonia, and fatal disseminated cerebral disease (for example, *Chaetomium atrobrunneum* is an invasive and neurotropic species) (Abdel-Azeem 2020). Therefore, it is extremely important to consider the pathogenic capacity of the fungus previously to choose it as BCA. However, pathogenic potential in humans is not the only aspect to considerate: it is important to square up if the antagonist can cause disease in animals or target plants, in addition to be effective against the phytopathogen. Although is rare, *Chaetomium* members have been reported as phytopathogens (Alam et al. 2021). In this sense, is important to assess the pathogenic potential of *Chaetomium* selected strains on target plant before, with previous *in vivo* experiments in healthy plants.

The poor information of *Chaetomium* biodiversity in Spanish soils, and especially in vineyards, makes necessary to investigate the role of this genera in *Vitis vinifera* and the entire ecosystem of the vineyard, and to assess if its biocontrol potential can be interesting taking into account the whole relationship. However, it has been illustrated that autochthonal microbiome from vineyards contain potentially biocontrolling strains against grapevine's cryptogamic diseases from *Chaetomiaceae* family, and that their different antagonistic mechanisms can provide to the agriculture new strategies for developing substitutes to chemical fungicides.

Conclusions

60 strains from *Chaetomiaceae* fungal family have been isolated from 17 different crop soil's samples. From these, 48 strains have been identified, both phenotypic and molecularly -using *tub*2 as DNA barcode-, and 12 are still unidentified. 20 isolates come from *Vitis vinifera* crops, which include *Arcopilus cupreus, Chaetomium carinthiacum, Arxotrichum succineum, Collariella bostrychodes, Collariella carteri, Chaetomium globosum, Dicotomophilus subfunicola, Chaetomium cochliodes, Chaetomium*

atrobrunneum, Chaetomium indicum, Chaetomium subfunicola, Botyotrichum murorum and *Amesia atrobrunnea.*

Chaetomium and *Chaetomium*-like species are interesting as biocontrol agents, not only because of their metabolites but also for their capacity of mycoparasitism and direct competition. None of them is especially effective against *Diplodia seriata*, but *C. globosum* and *C. cochliodes* are especially detachable against *Didymella glomerata,* inhibiting 62,67% the mycelial growth at the 14th day of incubation.

In relation to the antibiotic activity, none of the tested *Chaetomiaceae* strains have shown antibacterial activity against the tested bacteria, which include *Aeromonas* spp., *Escherichia coli, Serratia marcenses, Enterococcus faecalis* and *Klebsiella oxytoca.* The lack of results is probably due to the time and conditions of liquid culture, along with the experiment's design, instead of a missing antibacterial activity.

In conclusion, autochthonal microbiome of vineyards contain potential biocontrol agents, including *Chaetomium* and *Chaetomium*-like species, and is interesting to consider them in order to substitute chemical products used in agriculture to treat cryptogamic diseases.

Future perspectives

First, identifying all isolated strains must be done in order to complete the study. In addition, phylogenetic analysis of those strains that could be new species is a next step of this work.

3 previously reported as BCAs *Chaetomiaceae* species have been assessed for their use against cryptogamic diseases in viticulture. However, other of the isolated species could have biocontrol potential. Future studies should investigate the antagonistic capacity of the rest of isolated species. Another step is to assess the capacity of all isolated strains against more grapevine pathogens, with the objective of finding the most polivalent strain of *Chaetomium.*

Secondly, the improvement of the failed experiments must be done: commercial divided plates must to be employed in dual culture to determinate the volatile compounds inhibition and longer liquid cultures to obtain secondary metabolites is necessary to better determine the antibiotic activity of the strains. Also, a wider range of bacteria and soil bacteria have to be employed in agar diffusion test.

In addition, cryptogamic diseases are not exclusive from viticulture and grapevines but are a problematic of all crops. As is shown in **Table 1s** of Supplementary material, *Chaetomiaceae* strains have been isolated from soil samples of several horticultural crops too. In this sense, the future of this work is to assay them against phytopathogens that affect horticultural crops.

In the other hand, extraction, isolation, and identification of secondary metabolites produced by the different species and strains is of extremely interest as *Chaetomium*'s metabolites show a wide range of valuable bioactivities. With the development of genetic engineering and recombinant DNA techniques, the biocontrol strategies will can be improved with the use of the isolated or produced bioactive metabolites (Thambugala et al. 2020; Adnan et al. 2019).

Finally, *in vivo* and *in situ* experiments have to be assessed in order to confirm the biocontrol activity of the fungi in fields and demonstrate its capacity of substitute chemical fungicides.

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Supplementary material

Table 1s. Isolated and identified *Chaetomiaceae* species of the study, with sample, isolation conditions, BLAST and GenBank information. In blue there are indicated those strains correctly identified, in orange those that need further analysis to identify them with security, in red those not identified, and in black those that could be new species (<98% of identity in BLAST's results).

Code	FMR	ID	BLAST % identity	Sequence length	GenBank accession number	Sample origin	Isolation conditions
Ch1	19689	***Arcopilus cupreus*** strain CBS 560.80	100%	396 pb	KX976926.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Garnatxa. "Solà" vineyard	Ph-PCA 25ºC
Ch2	19690	***Chaetomium carinthiacum*** strain C87	100%	387 pb	HM365299.1	"	"
Ch3	19691	***Chaetomium carinthiacum*** strain C87	100%	238 pb	HM365299.1	**Clos Mogador**. Central plant cover, Garnatxa. "Solà" vineyard	"
Ch4	19692	***Arxotrichum succineum*** *strain CBS 119769*	98,55%	421 pb	MK919400.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Garnatxa Negra. "L'hort" vineyard	"
Ch5	19693	***Chaetomium atrobrunneum*** *C64*	99,27%	421 pb	HM365294.1	**Clos Mogador**. Central plant cover (10 years), Garnatxa Negra. "L'hort" vineyard	Ph-DRBC 25ºC
Ch6	19694	***Arxotrichum succineum*** *strain CBS 119769*	98,55%	421 pb	MK919400.1	**Clos Mogador**. Rhizosphere *Vitis vinífera*. Centennial vineyard	Ph-PCA 25ºC
Ch7	19695	***Amesia atrobrunnea*** *strain CBS 250.75*	98,82%	437 pb	KX976917.1	"	"
Ch8	19696	***Arxotrichum succineum*** *strain CBS 119769*	98,73%	395 pb	MK919400.1	"	"
Ch9	19697	***Dicotomopilus subfunicola*** CBS 794.83	100%	168 pb	KX977013.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Syrah. "Obaga" vineyard	"
Ch10	19698	***Chaetomium carinthiacum*** strain C87	99,37%	159 pb	HM365299.1	"	Ph-DRBC 25ºC

Ch11*	19699	***Chaetomium globosum*** strain CBS 160.62	99,45%	181 pb	KT214742.1	“	Ph-PCA 25ºC
Ch12*	19700	***Chaetomium cochliodes*** strain CBS 155.52	99,75%	395 pb	JN256147.1	“	Ph-DRBC 25ºC
Ch13	19701	***Collariella bostrychodes*** strain CBS 163.73	98,60%	508 pb	KX976983.1	“	“
Ch14	19702	***Chaetomium funicola*** strain CBS 158.52	100%	321 pb	JF772461.1	**VerdCamp Fruits**. Rhizosphere *Quercus ilex*	“
Ch15	19703	***Chaetomium coarctatum*** strain CGMCC 3.14299	100%	251 pb	JN256194.1	“	“
Ch16	19704	***Chaetomium* sp.**	ND	ND	ND	**VerdCamp Fruits**. *Cucumis sativus* crops	Ph-PCA 25ºC
Ch17	19705	***Chaetomium funicola*** *CBS 158.52*	98,16%	432 pb	JF772461.1	“	“
Ch18	19706	***Chaetomium funicola*** *CBS 158.52*	100%	402 pb	JF772461.1	“	“
Ch19	19707	***Collariella bostrychodes*** strain CBS 163.73	100%	121 pb	KX976983.1	“	“
Ch20	19708	***Chaetomium* sp.**	ND	ND	ND	“	“
Ch21	19709	***Chaetomium globosum*** strain LC4145	96,27%	403 pb	KP336886.1	“	“
Ch22	19710	***Collariella bostrychodes*** strain DTO 319-C4	97,65%	385 pb	KX976985.1	**VerdCamp Fruits**. Rhizosphere *Prunus persica*	“
Ch23	19711	***Chaetomium* sp.**	ND	ND	ND	**VerdCamp Fruits**. Rhizosphere *Pinus pinea*	“
Ch24	19712	***Collariella bostrychodes*** strain CBS 163.73	100%	367 pb	KX976983.1	**VerdCamp Fruits**. *Citrullus lanatus* crops	“
Ch25	19713	***Chaetomium* sp.**	ND	ND	ND	**VerdCamp Fruits**. Rhizosphere *Pinus pinea*	“
Ch26	19714	***Chaetomium biapiculatum*** *strain LC3853*	99,50%	403 pb	KP336857.1	“	“
Ch27	19715	***Arcopilus cupreus*** strain CBS 560.80	98,46%	389 pb	KX976926.1	**Clos Mogador**. Central plant cover, Garnatxa. “Solà” vinyeard	Ph-DRBC 25ºC
Ch28*	19716	***Arcopilus cupreus*** strain CBS 560.80	97,90%	441 pb	KX976926.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Garnatxa. “Solà” vinyeard	“

Ch29	19717	***Arxotrichum succineum*** strain CBS 119769	99,25%	408 pb	MK919400.1	“	“
Ch30	19718	***Chaetomium carinthiacum*** strain C87	100%	387 pb	HM365299.1	**Clos Mogador**. Central plant cover. “Obaga” vinyeard	“
Ch31	19719	***Arcopilus cupreus*** strain CBS 560.80	98,40 %	375 pb	KX976926.1	“	Ph-DRBC 25ºC
Ch32	19720	***Chaetomium carinthiacum*** strain C87	99,75%	397 pb	HM365299.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Syrah. “Obaga” vinyeard	Ph-PCA 25ºC
Ch33	19721	***Chaetomium subfunicola*** strain C37	99,74%	392 pb	HM365300.1	“	“
Ch34	19722	***Arcopilus cupreus*** strain CBS 560.80	98,52%	406 pb	KX976926.1	**Clos Mogador**. Rhizosphere *Vitis vinífera*. Centennial vinyeard	“
Ch35	19723	***Chaetomium indicum*** strain C2	99,75 %	396 pb	HM365301.1	“	“
Ch36	19724	***Chaetomium* sp.**	ND	ND	ND	“	“
Ch37	19725	***Arxotrichum succineum*** strain CBS 119769	99,21%	378 pb	MK919400.1	“	
Ch38	19726	***Chaetomium* sp.**	ND	ND	ND	**VerdCamp Fruits**. Rhizosphere *Vicia fava*	Ph-PCA 25ºC
Ch39	19727	***Chaetomium* sp.**	ND	ND	ND	**VerdCamp Fruits**. Rhizosphere *Pinus pinea*	“
Ch40	19728	***Chaetomium cancroideum*** CBS 154.52	98,35%	427 pb	JX215349.1	**VerdCamp Fruits**. Rhizosphere *Quercus ilex*	Ph-DRBC 25ºC
Ch41	19729	***Chaetomium* sp.**	ND	ND	ND	**VerdCamp Fruits**. Rhizosphere *Pinus pinea*	“
Ch42	19730	***Chaetomium* sp.**	ND	ND	ND	**VerdCamp Fruits**. Rhizosphere *Quercus ilex*	“
Ch43	19731	***Chaetomium* sp.**	ND	ND	ND	**Clos Mogador**. Rhizosphere *Vitis vinifera* Syrah. “Obaga” vinyeard	
Ch44	19732	***Chaetomium* sp.**	ND	ND	ND	Johnson Su’s compost	Ph-DRBC 25ºC
Ch45	19733	***Collariella carteri*** CBS 128.85	100%	395 pb	KX976989.1	**Clos Mogador**. Central plant cover (10 years), Garnatxa Negra. “L’hort” vinyeard	“
Ch46	19734	***Collariella carteri*** CBS 128.85	100%	397 pb	KX976989.1	**VerdCamp Fruits**. Rhizosphere *Curcubita maxima. “*Salis”	Ph-PCA 15ºC
Ch47	19735	***Chaetomium* sp.**	ND	ND	ND	**VerdCamp Fruits**. Rhizosphere *Curcubita maxima.* “Alzina”	“

Ch48	19736	***Arcopilus cupreus*** *strain CBS 560.80*	98,40 %	375 pb	KX976926.1	**Clos Mogador**. Central plant cover. “Obaga” vineyard	Ph-DRBC 25ºC
Ch49	19737	***Dichotomopilus subfunicola*** *CBS 794.83*	99,47%	383 pb	KX977013.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Garnatxa Negra. “L’hort” vineyard	Ph-PCA 25ºC
Ch50	19888	***Chaetomium biapiculatum*** LC3852	99,23%	392 pb	KP336856.1	**VerdCamp Fruits.** Rhizosphere *Brassica oleracea* i *Foeniculum vulgare*	Ph-DRBC 15ºC
Ch51	19889	***Chaetomium* sp.**	ND	ND	ND	“	“
Ch52	19890	***Collariella bostrychodes*** DTO 319-C4	99,74%	388 pb	KX976985.1	“	Ph-PCA 15ºC
Ch53	19891	***Talaromyces* sp.**	100%	389 pb	MK451254.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Garnatxa. “Carretera” vineyard	Ph-PCA 25ºC
Ch54	19892	***Arcopilus cupreus*** strain CBS 560.80	98,77%	406 pb	KX976926.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Carinyena. “Bienvenido” vineyard	“
Ch55	19893	***Arcopilus cupreus*** strain CBS 560.80	98,06%	411 pb	KX976926.1	**Clos Mogador**. Rhizosfere Syrah and Garnatxa. “Obaga” vineyard	Ph-DRBC 25ºC
Ch56	19894	***Chaetomium carinthiacum*** strain C87	97,58%	206 pb	HM365299.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Carinyena. “Bienvenido” vineyard	Ph-DRBC 15ºC
Ch57	19895	***Chaetomium cuniculorum*** CBS 121.57	99,75%	410 pb	KP900709.1	**VerdCamp Fruits**. Rhizosphere *Curcubita maxima. “*Salis”	“
Ch58	19896	***Chaetomium undulatum*** C78	99,21%	381 pb	HM365279.1	“	Ph-PCA 25ºC
Ch59	19897	***Botyotrichum murorum*** CBS 173.68	100%	404 pb	KX976934.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Garnatxa. “Carretera” vineyard	Ph-PCA 25ºC
Ch60	19898	***Arxotrichum succineum*** strain CBS 119769	99,25%	402 pb	MK919400.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Garnatxa. “Carretera” vineyard	Ph-PCA 25ºC
Ch61	19899	***Arxotrichum succineum*** strain CBS 119769	96,30%	107 pb	MK919400.1	**Clos Mogador**. Rhizosphere *Vitis vinifera* Carinyena. “Bienvenido” vineyard	Ph-PCA 15ºC

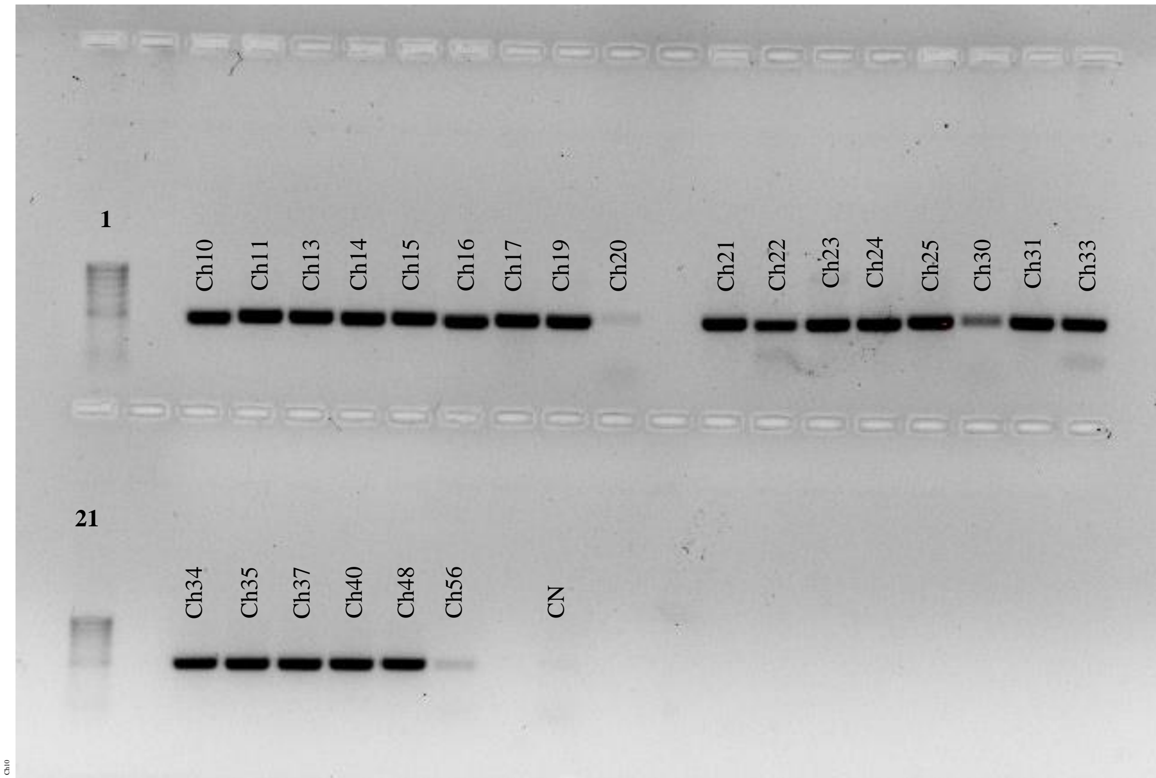
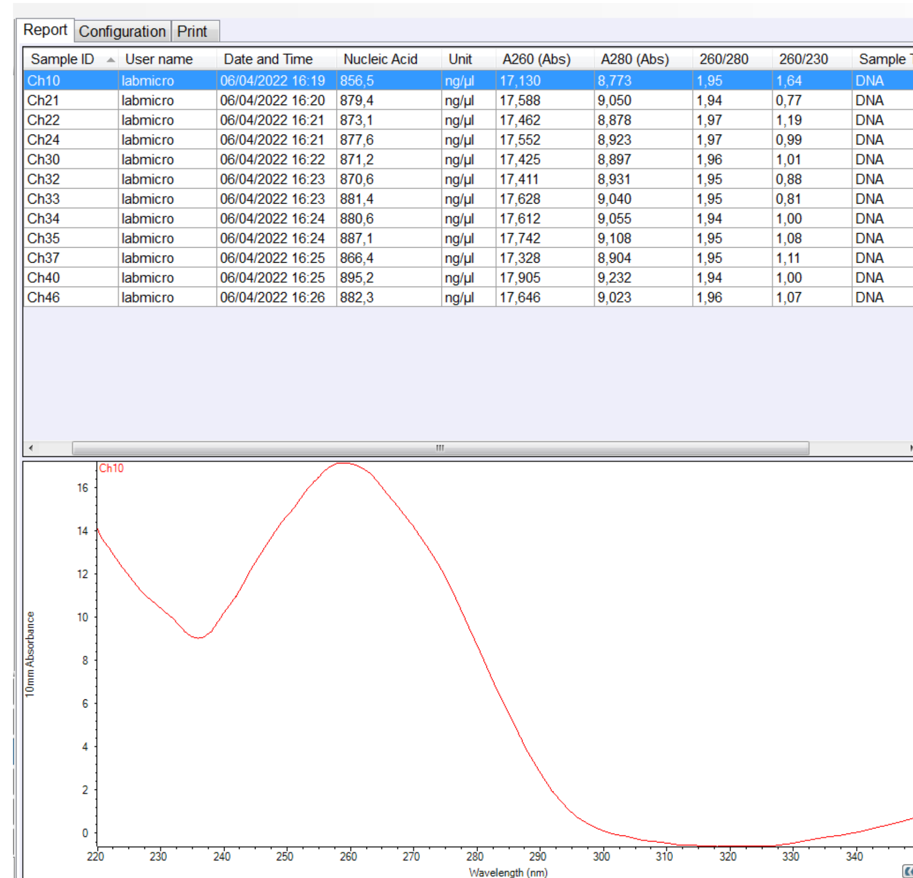


Figure 1s. Resulting electrophoresis gel from amplification of *tub*2 barcode of different *Chaetomium* strains. In lanes 1 and 21, molecular weight sizer (PCR Sizer 100 bp DNA Ladder, Norgen Biotek Corp, Thorold, Canada). Other lanes tagged in image. In lane 30, negative control, corresponding to PCR mix without template.



Sample ID	User name	Date and Time	Nucleic Acid	Unit	A260 (Abs)	A280 (Abs)	260/280	260/230	Sample T
Ch10	labmicro	06/04/2022 16:19	856,5	ng/µl	17,130	8,773	1,95	1,64	DNA
Ch21	labmicro	06/04/2022 16:20	879,4	ng/µl	17,588	9,050	1,94	0,77	DNA
Ch22	labmicro	06/04/2022 16:21	873,1	ng/µl	17,462	8,878	1,97	1,19	DNA
Ch24	labmicro	06/04/2022 16:21	877,6	ng/µl	17,552	8,923	1,97	0,99	DNA
Ch30	labmicro	06/04/2022 16:22	871,2	ng/µl	17,425	8,897	1,96	1,01	DNA
Ch32	labmicro	06/04/2022 16:23	870,6	ng/µl	17,411	8,931	1,95	0,88	DNA
Ch33	labmicro	06/04/2022 16:23	881,4	ng/µl	17,628	9,040	1,95	0,81	DNA
Ch34	labmicro	06/04/2022 16:24	880,6	ng/µl	17,612	9,055	1,94	1,00	DNA
Ch35	labmicro	06/04/2022 16:24	887,1	ng/µl	17,742	9,108	1,95	1,08	DNA
Ch37	labmicro	06/04/2022 16:25	866,4	ng/µl	17,328	8,904	1,95	1,11	DNA
Ch40	labmicro	06/04/2022 16:25	895,2	ng/µl	17,905	9,232	1,94	1,00	DNA
Ch46	labmicro	06/04/2022 16:26	882,3	ng/µl	17,646	9,023	1,96	1,07	DNA

Figure 2s. Nanodrop 2000 software's interface. Results of the samples' DNA quantification after amplification. First column corresponds to sample identification, 4th column to DNA concentration expressed in ng/µl and 6th column refers to quality (absorbance relation A260/A280). DNA quality must be between 1,9 and 2,1 to be acceptable. At the bottom there is the absorbance graphic of Ch10 strain (sample's absorbance in relation to the wavelength in nanometers).