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FROM CONTINENTAL ANTARCTICA A PSYCHROTOLERANT STRAIN (CCFEE 5003) OF *LECANICILLIUM MUSCARIUM* THAT MYCOPARASITE FUNGI AND OOMYCETES

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Abstract

The psychrotolerant fungus Lecanicillium muscarium (CCFEE 5003), isolated in Continental Antarctica, was able to exert mycoparasitism in laboratory conditions (agar co-coltures), at different temperatures (from 5 to 28°C), against a wide array of filamentous fungi such as Mucor mucedo, M. plumbeus, Botrytis cinerea, Cladosporium cladosporoides, Aspergillus versicolor and Penicillium verrucosum. Mycoparasitism against oomycetes such as Pythium aphanidermatum and Phytophthora palmivora was also recorded, while its action against yeasts is still controversial. The mycoparasitism of this organism consists of various sequential steps leading to complete host disruption. Against fungi, parasitism was characterised by diffused penetration into the host mycelium. Against oomycetes, penetration was less evident even if contact between the two organisms was more intimate. Production of cell wall degrading enzymes such as chitinolytic and glucanolytic enzymes appeared strongly related to the mycoparasitic process.

Keywords: mycoparasitism, Lecanicillium muscarium, fungi, oomycetes, Continental Antarctica, cell wall degrading enzymes.

1. INTRODUCTION

Lots of eukaryotic microorganisms cause plant diseases and food spoilage with large economic losses due both to the diseases and to the control measures to be adopted. Among filamentous fungi, the genus *Botrytis* is known for its pathogenicity while species of *Mucor* are often involved in food spoilage [1,2]. Others, such as various Penicilli and Aspergilli, could contaminate cereals and produce toxins (i.e. ochratoxin A) [3,4].
A number of oomycetes belonging to *Pythium* and the closely related genus *Phytophthora* are known for the severe damages to very important crops (i.e. those belonging to *Solanum* and *Lycopersicon*). For instance, world-wide losses in potato production caused by *Phytophthora* species have been estimated to cost yearly several billion of U.S. dollars [5,6].
Due to undesirable side effects on human health and on the environment, the use of traditional chemical fungicides, pesticides, and food preservatives has been widely criticized. As a consequence, microorganisms and/or their products (i.e. cell wall degrading enzymes or antibiotics) have been studied in order to develop more safe alternatives to chemicals [1,2,6-8]. Many studies concerned *Trichoderma harzianum* a mycoparasitic fungus, producing a number of cell-wall degrading enzymes, that is commercially used as biological pest control agent [9-12]. Extracellular cell-wall degrading enzymes (i.e. chitinases and glucanases) have been demonstrated to exert a fundamental aggressive role in mycoparasitism disarranging the dynamic of the host hyphal growth and leading to lysis [13,14].
Lecanicillium muscarium is a well known entomopathogenic fungus [15-18]. It also exerts mycoparasitic activity against fungi involved in plant diseases [19-22]. Moreover, its potential as a biocontrol agent against some fungal plant pathogens has been demonstrated [21].

2. MATERIAL AND METHOD

In this short review the mycoparasitic role of an Antarctic psychrophilic strain (CCFEE 5003) of *Lecanicillium muscarium* (ex *Verticillium lecanii* [23-24]), against various moulds and oomycetes is described. Short comments are also given on its possible mycoparasitism against yeasts.

2.1. Mycoparasitism

Mycoparasitism could be defined as a parasitic action of a fungus (parasite) against another fungus (host) involving direct contact between the two fungi and resulting in host death, and nutrient absorption by the parasite. Mycoparasites produce cell wall-degrading enzymes (CWDE) which allow them to bore holes into other fungi and extract nutrients for their own growth. A lot of mycoparasites also produce antibiotics which may first weaken the fungi they parasitize. Actually, it is not very easy to define mycoparasitism since it depends upon the two organisms involved. Bruce et

al. [25]. described it as an antagonistic interaction between two fungi during which the aggressor makes intimate contact with the target organism before releasing hydrolytic enzymes to facilitate the degradation of the host cell wall. Others [26-28] gave a more strict definition relating the mycoparasitism to the development of typical actions such as coiling around and penetration into the host. Production of lytic enzymes and/or antibiotics was also mentioned. Sometime, mycoparasitism is simply defined as the attack of a fungus against other fungi [29, 30].
Most authors, however, agree that a firm contact between the two organism must occur [25-28].
Sometime, mycoparasites such as *Trichoderma* spp. establish inhibitory interactions with other organisms producing extracellular metabolites and/or volatile compounds with no physical contact between the two organisms [27].

2.2. Mycoparasitism of Lecanicillium muscarium CCFEE 503 against filamentous fungi and yeasts

Already during the very early studies, the production of CWDE such as chitinases and proteases was evident as major extracellular hydrolytic competence of this organism [1,31,32]. Later on, it was confirmed that this fungus is a very strong producer in particular of chitinases and glucanases when grown on various typologies of chitins and glucans [6]. Thus, presence of high levels of CWDE led to suppose a mycoparasitic role of the fungus.
Hence *L. muscarium* was tested in vitro for its mycoparasitic activity against various filamentous fungi all involved in food spoilage, plant pathogenesis or production of mycotoxins. The organisms tested, in dual cultures, as possible hosts for *L. muscarium* were: *Mucor plumbeus*, *Cladosporium cladosporoides* (Figure 1), *Aspergillus versicolor*, *Penicillium verrucosum* [1], *Mucor mucedo* and *Botrytis cinerea* [6].

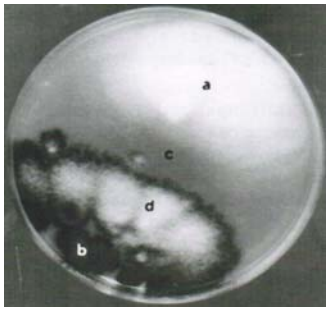

Figure 1. Co-culture of *L. muscarium* (a) and *C. cladosporioides* (b). c = inhibition zone between the 2 fungi; d = overgrowth of *L. muscarium* on *C. cladosporioides* [1].

The Antarctic fungus mechanism of parasitism followed a typical sequence of events. All plate co-cultures of *L. muscarium* and a host fungus showed common features. The tested host fungi grew well until they reached a distance of 3-10 mm from the colonies of *L. muscarium* thus entering a so called "contact zone" or "inhibition zone" [1].
Then, all host organisms stopped their growth while mycelial modifications, never occurring in pure cultures of the same organisms, such as branching, damages, presence of strange structures and sometime protoplast formation became evident. This suggested that *L. muscarium* released into the medium some diffusible and inhibiting factors (i.e. CWDE and/or antibiotics). This is typical of mycoparasitic fungi such as *Trichoderma* spp. [25,27,28] or *Verticillium* spp. [22, 32].
However, after some time, *L. muscarium* began to get in contact with the host and, shortly thereafter, to overgrown on it (Figure 2).

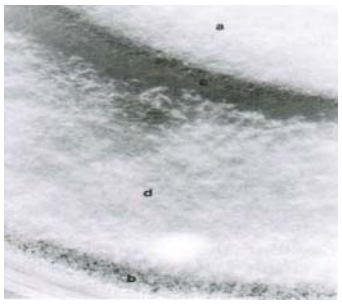

Figure 2. Co-culture of *L. muscarium* (a) and *m. plumbeus* (b), detail. c = inhibition zone between the 2 fungi; d = overgrowth of *L. muscarium* on *M. plumbeus* [1].

Samples of the contact zones from agar co-cultures were observed under light microscopy and SEM to understand the mycoparasitism mechanism at the microscopic level.
In the first phases firm attachment of the parasite to the host mycelium was recorded. This included coiling and mechanical pressure on the host mycelium (figure 3). Coils are less regularly developed than in other fungi such as *T. harzianum*.

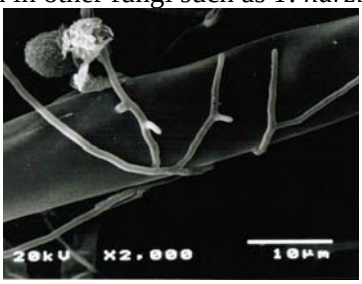

Figure 3. Co-culture of *L. muscarium* and *M. plumbeus*: early phase of the parasitism. The tiny mycelium of *L. muscarium* entered in contact with the host bigger mycelium starting to coil around it and to exert mechanical pressure [1].

3. RESULTS AND DISCUSSIONS

Then, probably aided by the production of cell-wall degrading enzymes (Figure 4), the parasite penetrated into the host cell (Figure 5) [1,6].

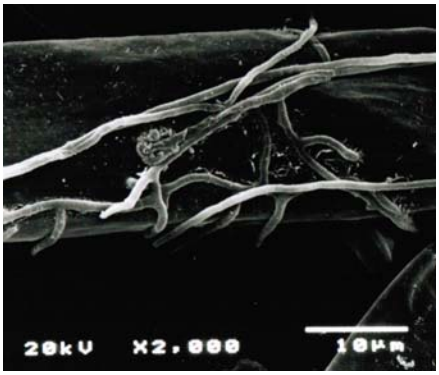

Figure 4. Co-culture of *L. muscarium* and *M. plumbeus*: intermediate phase of the parasitism. Contact with the host mycelium is more evident. Coils exert more mechanical pressure and it is possible to see host mycelium degradation close to the parasite hyphal tips [1].

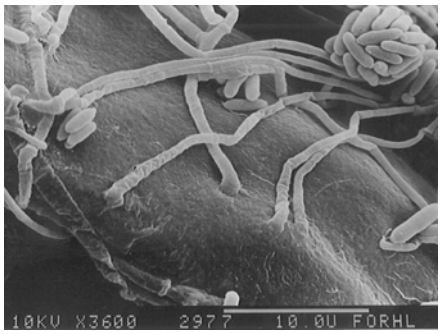

Figure 5. Co-culture of *L. muscarium* and *M. mucedo*: intermediate phase of the parasitism. The parasite penetrate into the host mycelium. Sporulation also occurs.

Similar mechanism is reported by Askary (1996). In all cases, at the late stages of the interaction, *L. muscarium* caused complete destruction of the host that appeared strongly deflated, depressed and invaded by the parasite (Figures 6, 7).

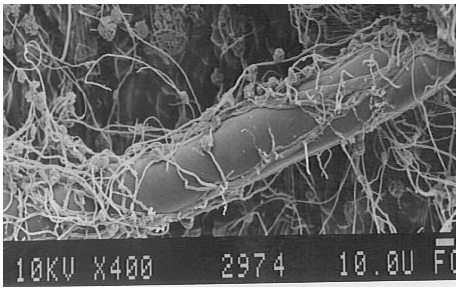

Figure 6. Co-culture of *L. muscarium* and *M. mucedo*: late phase of the parasitism. Contact with the host mycelium is very intense [6].

Abundant sporulation of *L. muscarium* was always observed during the whole process (Figures 5, 6).

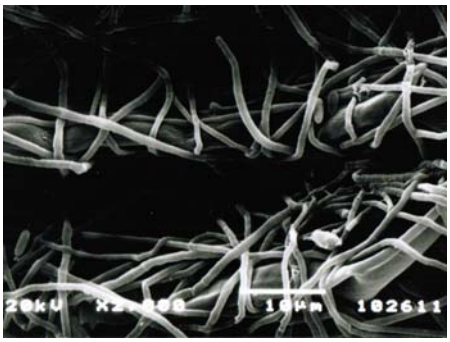

Figure 7. Co-culture of *L. muscarium* and *M. plumbeus*: final phase of the parasitism. Contact with the host mycelium is extreme. The host mycelium is overwhelmed by the parasite and appeared completely deflated [1].

Since filamentous fungi have chitin as major cell wall component, the high level of chitinolytic enzymes produced by *L. muscarium* widely justifies the strong aggressive behavior against these organisms. In fact, the purified chitinolytic enzymes used on the above mentioned test fungi caused similar effects of mycelium damages, protoplast formation and so on [1,6].

As for the action of the Antarctic fungus on yeasts, preliminary results (Fenice et al., unpublished results) showed that, on co-cultures (*L. lecanii* and *candida vinaria*), the fungus behavior was very similar to that described above. After some time it was able to overgrow on the yeast (Figure 8).

Figure 8. Co-culture of *L. muscarium* and *C. vinaria*. The white mycelium of the fungus overgrown on the yeast.

However, microscopic observations showed that the yeast was not completely overwhelmed by the fungus.

Apparently no contact was recorded between the fungus and the yeast but many yeast cells appeared bigger than usual and strongly damaged.

In addition, yeast cells were morphologically modified and traces of cell lysis were evident (Figure 9). Very likely, the fungus released some

CWDE affecting the yeasts but their concentration was not high enough. Further experiments are needed to understand possible parasitic role of *L. muscarium* against yeasts.

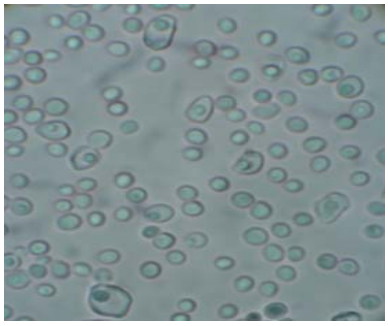

Figure 9. Cells of *C. vinaria* grown in co-cultures with *L. muscarium*. Most of them are bigger than usual and damaged, their cell wall is not regular. Traces of cell lysis are evident.

Mycoparasitism of Lecanicillium muscarium CCFEE 503 against oomycetes

Oomycetes do dot have chitin on their cell wall that has glucans as main components.

Lecanicillium muscarium was tested against *Pythium aphanidermatum* and *Phytophthora palmivora*. Mechanisms of mycoparasitism against these organisms appeared quite different from that recorded for fungi. No evident penetration into the host cell was recorded but only a very firm adhesion and possibly mechanical pressure occurred. The mycelium of *L. muscarium* appeared sometime almost fused with that of the host (Figure 10). In all cases, at the late stages of the interaction, *L. muscarium* caused complete destruction of the host that appeared strongly deflated, depressed and invaded by the parasite. Intense sporulation occurred in this case as well (Figure 10) [6].

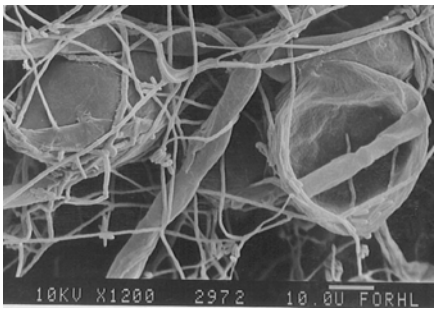

Figure 10. Co-culture of *L. muscarium* and *P. palmivora*: late phase of the parasitism. Contact with the host mycelium is very intense, host mycelium and fruiting bodies completely deflated. *L. muscarium* sporulation occurred [6].

Mycoparasitism appeared to be particularly effective here being exerted also against *P. aphanidermatum* that is known as itself a mycoparasite [29].

All the mycoparasitic interactions with fungi and oomycetes were recorded in a wide range of temperature (from 5 to 28 °C) [1,6].

4. CONCLUSIONS

Mycoparasitism is a very interesting interaction, occurring between two eukaryotic microorganisms, leading to the disruption of one of them. This natural occurring phenomenon is exploited for the biological control of pathogens such fungi and oomycetes that affect food and important crops. Most of the studies had regarded the well known fungus *Trichoderma harzianum*. However, as already reported in the early 90ies [34], its activity is sometime limited due to its scarce activity at low temperature. Thus, its application in cold environments or seasons could be a problem.

Lecanicillium muscarium CCFEE 5003 is a very powerful psychrotolerant mycoparasite acting against both pathogenic fungi and oomycetes. Its activity is very high at low (5°C) temperature, also. Therefore, this organism could be proposed as a very promising new biocontrol agent. However, till now all the study had been carried out in co-cultures at laboratory scale. In order to avoid ecological problems, prior to release this Antarctic organism in other environments, field experiments under controlled conditions (i.e. greenhouses) must be carried out.

5. ACKNOWLEDGMENTS

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