In Vitro Nematophagous Activity of Fungi Isolated from Soil and Some Heteroderidae (Nematoda: Tylenchida)

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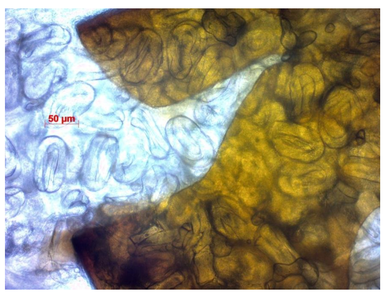
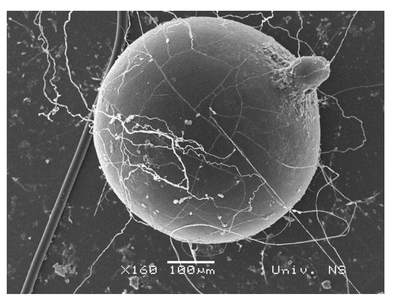
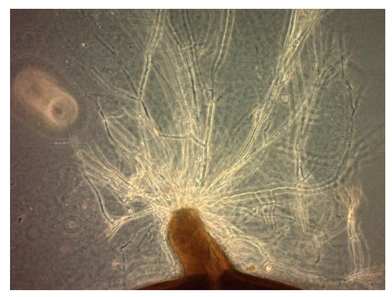
Cyst nematodes which belong to family Heteroderidae are the most recognized and economically important nematodes worldwide, especially the ones from genera *Globodera* and *Heterodera*. Potato cyst nematode, *Globodera rostochiensis* is, together with *G. pallida*, one of the most important potato parasites. The external and internal area of a cyst harbor many fungal and bacterial species competing for substrate. Thus, the cyst can be considered as a specific "microcosm" containing numerous microorganisms. Some of these microbes could be potential biocontrol agents.



A broken cyst of *Globodera rostochiensis* with visible eggs and larvae inside



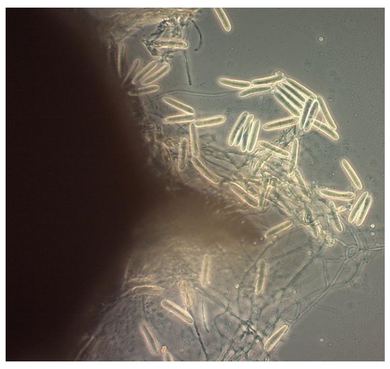
Fungal colonization of the cyst surface


Fungal hyphae associated to the the internal of a *G. rostochiensis* cyst, coming out from the neck

Methods and Materials

Tests were performed *in vitro* in order to evaluate the capacity of different fungal strains to attack cysts, eggs or juveniles of *Globodera rostochiensis*. The strains are part of the Mycology Laboratory fungal collection (University of Pavia) and were isolated from the following samples, collected in different areas: soil from organic potato crop (Emilia Romagna, Italy), soil from the Integral Natural Reserve "Bosco Siro Negri" (Lombardy, Italy), cysts of *G. rostochiensis* (Emilia Romagna, Italy), cysts of *G. pallida* (Kladnica, Serbia), cysts of *Heterodera glycines* (Lombardy, Italy).

These are some of the species used in the tests


Cylindrocarpon destructans isolated from cysts of *H. glycines*

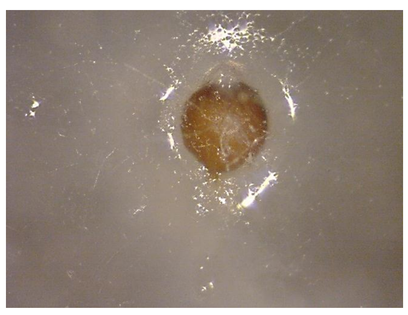

Paecilomyces lilacinus from soil


Verticimonosporium verticale from soil

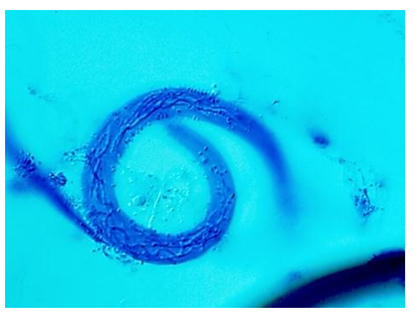

Pochonia chlamydosporia var. *chlamydosporia* from *G. rostochiensis* cysts

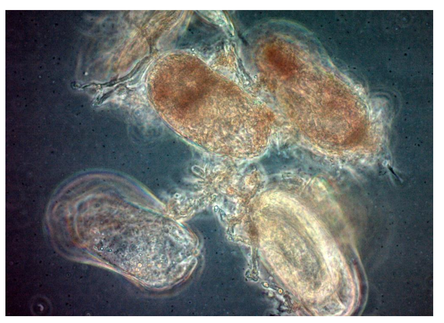

Pochonia chlamydosporia var. *catenulata* from soil

Each fungal species was cultivated in plates with cysts of *G. rostochiensis,* distributed on the water agar surface. Cysts were washed 10 times with a solution of sterilized distilled water with penicillin-G, streptomycin, aureomycin at 50 ppm, and neomycin and chloramphenicol at 100 ppm. Controls were represented by water agar plates where only cysts were distributed on the surface. After a period of 10-20 days at 25°C the plates were controlled every week for two months and cysts, eggs and juveniles' condition was considered. The presence of colonized cysts, eggs and juveniles were counted and compared with the total observed.


A *G. rostochiensis* cyst on the agar surface


A *G. rostochiensis* juvenile crawling on agar surface

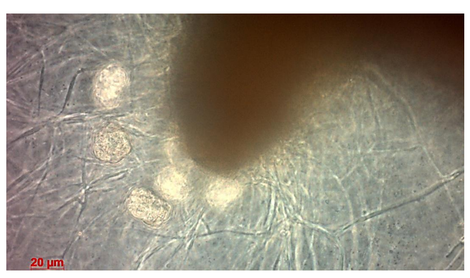

A juvenile colonized by *P. chlamydospora* var. *catenulata*


Eggs colonized by *Verticimonosporium verticale*

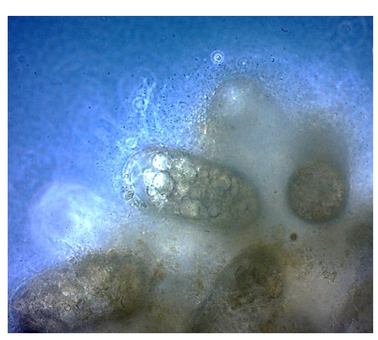
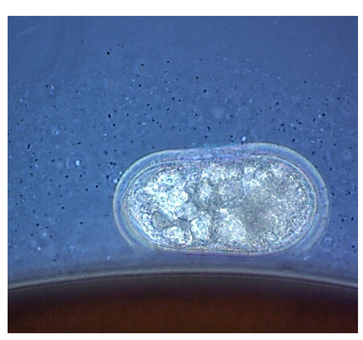
Main Results

The following table shows the % of the colonized cysts, eggs and juveniles. All the tested strains were able to grow on cysts but this did not have effect on the vitality of juveniles. Some of the fungal strains, such as *Cylindrocarpon destructans, Paecylomyces lilacinus*, *Verticimonosporium verticale*, *Fusarium oxysporum* were not detrimental to the host. The best results were obtained in the tests where *Pochonia chlamydosporia* was inoculated. No differences were recorded between *P. chl.* var. *chlamydosporia* and *P. chl.* var. *catenulata*. The fungus was not able to colonize the juveniles but colonized cysts filling up the eggs with their chlamydospores inhibiting the juvenile development. Experiments are in progress to verify the ability of killing *Globodera rostochiensis* juveniles by a consortium made of *P. chlamydosporia* and a nematode attacking fungus such as *Lecanicillium lecanii* or *Arthrobotrys oligospora*.

	Colonized Cysts	Colonized Eggs	colonized dead Juveniles	alive juveniles
Cylindrocarpon destructans	60%	0%	0%	100%
Paecilomyces lilacinus	40%	0%	0%	100%
Verticimonosporium verticale	10%	1%	0%	100%
Pochonia chlamydosporia var. *chlamydosporia*	100%	100%	0%	50%
Pochonia chlamydosporia var. *catenulata*	100%	100%	0%	50%
Fusarium oxysporum	100%	0%	0%	100%



Chlamydospores produced by *P. chlamydosporia* growing into and on to a *Globodera rostochiensis* cyst




Eggs of *Globodera rostochiensis* filled up of chlamydospores of *Pochonia chlamydosporia*

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