The ovicidal activity of fungi *Pochonia chlamydosporia* and *Paecilomyces lilacinus* on *Taenia saginata* eggs in laboratory trial

Atividade ovicida dos fungos *Pochonia chlamydosporia* e *Paecilomyces lilacinus* sobre ovos de *Taenia saginata* em condições laboratoriais

RIALA6/1272

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Recebido: 17.01.2010 – Aceito para publicação: 10.06.2010

RESUMO

A atividade ovicida (efeito tipo 3) dos fungos *Pochonia chlamydosporia* (isolados VC1 e VC4) e *Paecilomyces lilacinus* (PL1) sobre os ovos de *Taenia saginata* foi investigada, em condições laboratoriais. Os ovos de *T. saginata* foram colocados em placas de Petri contendo o meio agar-água 2% (AA 2%) com os isolados fúngicos e, também, em placas de Petri sem fungos, como controle. A atividade ovicida desses fungos foi avaliada depois de 5, 10 e 15 dias de incubação. Ao final do experimento, os fungos *P. chlamydosporia* (VC1 e VC4) e *P. lilacinus* (PL1) demonstraram atividade ovicida ($p<0,05$) quando comparados com o controle. Contudo, aos 15 dias de incubação o fungo *P. lilacinus* demonstrou maior atividade ovicida (efeito do tipo 3) em relação ao *P. chlamydosporia*. Este estudo demonstrou que os fungos *P. chlamydosporia* (VC1 e VC4) e *P. lilacinus* (PL1) possuem a capacidade de destruir os ovos de *T. saginata* e, portanto, podem ser considerados como potenciais candidatos no controle biológico deste cestoda.
Palavras-chave. Fungos nematófagos, *Pochonia chlamydosporia*, *Paecilomyces lilacinus*, Controle biológico, *Taenia saginata*.

ABSTRACT

The ovicidal activity (type 3 effect) of fungi *Pochonia chlamydosporia* (VC1 and VC4 isolated) and *Paecilomyces lilacinus* (PL1) on *Taenia saginata* eggs was compared in an experimental trial. *T. saginata* eggs were fixed onto Petri dishes containing 2% water-agar (2% WA) and fungal isolates, and onto Petri dishes without fungus as control. The ovicidal activity of these fungi was evaluated after being incubated for 5, 10 and 15 days. From the beginning of the interaction to the end of the trial, both *P. chlamydosporia* (VC1 and VC4) and *P. lilacinus* (PL1) fungi demonstrated ovicidal activity ($p<0,05$) when compared to the control. However, after 15 days of incubation the *P. lilacinus* fungus showed higher ovicidal activity (effect of type 3) when compared to *P. chlamydosporia*. This study showed that both fungi *P. chlamydosporia* (VC1 and VC4) and *P. lilacinus* (PL1) are capable in killing eggs of *T. saginata*, therefore these fungi might be considered as potential candidates for biological control of this cestoda.
Key words. Nematophagous fungi, *Pochonia chlamydosporia*, *Paecilomyces lilacinus*, Biological control, *Taenia saginata*.

INTRODUCTION

Helminth infections are still an important problem for public health, especially regarding zoonosis[1]. Among these infections, there is taeniasis/cysticercosis complex, affecting about 45 million of people worldwide[2].

The taeniasis/cysticercosis complex developed by *Taenia saginata* has a cosmopolitan distribution and is widespread in most of countries where there is cattle raising. However, Brazil is in a privileged situation in the world scenario in bovine raising, since it is the biggest producer of commercial cattle in the world[3].

The *T. saginata* cycle implies two hosts, one definitive and one intermediate, and a free phase. The only definitive host is human, in whose small intestine it lodges. In its turn, intermediate *T. saginata* host is bovine. Three phases regarding the population of these parasites are known: adult in the definitive host, eggs on environment and larval phase in the intermediate host[4]. According to Gemmell and Lawson[5] some factor might favorably contribute for the dispersion of *T. saginata* eggs in the environment and among them is the fecal contamination of soil.

Humans can be infected through the ingestion of raw or undercooked meat with cysticerci and the disease, taeniasis, can present itself in an asymptomatic way, but in some cases changes in appetite, nausea, vomiting, abdominal pains, diarrheas, loss of weight, irritability and fatigue can coexist[6, 7].

To fight the taeniasis/cysticercosis complex, several measures can be employed, but the main strategy consists in the interruption of the parasite life cycle[8]. On the other hand, one of the main factors that condition the development and permanence of this parasite's eggs in the soil and its destruction are the antagonistic organisms, such as ovicidal nematophagous fungi[9]. However, studies on the natural processes of destruction of geohelminth eggs are still in initial stages, but they represent an interesting alternative that, used in combination other prophylactic measures, can contribute to the control of species of epidemiological importance.

Nematophagous fungi comprehend different type of fungi, divided into predators, endoparasites and ovicidal, are cosmopolitan, occurring in natural soils, croplands and all types of organic material in decomposition[10]. The eggs of the parasite fungi group consists of saprophytic and, therefore, are independent from the presence of helminth eggs in the soil for its survival, in addition of being easily isolated and cultured in lab. These fungi colonize the egg content, resulting in its destruction[11, 12].

Among the promising species of this group, there is fungi *Pochonia chlamydosporia* and *Paecilomyces lilacinus*. These fungi have wide distribution and have been successfully used in lab conditions in the biological control of several gastrointestinal parasites of nematode genus[13, 14]. However, its ovicidal activity (type 3 effect) was never compared in lab conditions over eggs of gastrointestinal helminth parasites[15].

Araújo et al[10] mention that the biological control, in practice, does not act on internal stages of the parasite. It acts on intermediate hosts, paratene, vectors and infective stages (infective larvae and embryonated eggs), thus decreasing the source of infection to final hosts and causing less negative effects in the environment.

The objective of this research was to compare the ovicidal activity (type 3 effect) of ovicidal fungi *Pochonia chlamydosporia* (VC1 and VC4 isolated) and *Paecilomyces lilacinus* (PL1) over eggs of *Taenia saginata*.

MATERIAL AND METHODS

Fungi

Two isolates of the nematophagous fungus *P. chlamydosporia* (VC1 and VC4) and one isolate of the nematophagous fungus *P. lilacinus* (PL1) were used. These isolates are from soil of Brazil, in the location of Viçosa, in Minas Gerais, latitude 20°45'20"S, longitude 42°52'40" W, at 649 m from sea level. Fungi were kept in assay tubes at 4ºC with corn-meal-agar 2% and in dark for 10 days.

After the growth of isolates, new culture discs of 4 mm diameter were transferred to Petri dishes 9.0 cm diameter with 20 mL of 2% water-agar (2% WA) for 10 days.

Obtaining *Taenia saginata* eggs

T. saginata eggs were recovered from proglottids dissecting of an adult specimen spontaneously donated by a human patient diagnosed with taeniasis. Following, the eggs were morphologically analyzed as for integrity on optical microscope in 10x objective[16].

Assay

T. saginata eggs were fixed on the surface of Petri dishes of 9.0 cm diameter with the 2% WA medium with grown fungal isolates for 10 days and without fungus as control, being 25 repetitions per each group performed.

In treatments, each plate had a thousand *T. saginata* eggs with only one fungal isolate. On intervals of 5, 10 and 15 days, one hundred eggs were removed from each plate with the isolate and control without fungus according to the technique described by Araújo et al[17], being assessed in 40x objective according to parameters established by Lysek et al[18]: type 1, physiological effect without morphological harm to the eggshell, where hyphae are noticed adhered to the shell; type 2, lytic effect with morphological change of egg shell and embryo, without hyphae penetration through the shell, and type 3, lytic effect with morphological change of embryo and shell, and also hyphae penetration and internal colonization of the egg.

Data of each studied interval were submitted to nonparametric Friedman's test with 5% of probability[19]. Statistic analyses were performed with aid of BioEstat 3.0 software.

RESULTS

Percentage results of type 1, 2 and 3 effects presented by fungi *P. chlamydosporia* (VC1 and VC4) and *P. lilacinus* (PL1) throughout the experimental test of 5, 10 e 15 days are represented in Table 1.

Isolate VC1 had type 1, 2 and 3 effects throughout the studied intervals. For ovicidal activity (type 3 effect) this isolate had percentage values of 12.8%, 18.2% and 9.7% at 5, 10 and 15 days, respectively. In the same way, isolate VC4 had all type 1, 2 and 3 effects and at the end of the experimental test showed percentage of ovicidal activity of 2.3%, 7.0% and 8.0% in the same studied intervals.

Fungus *P. lilacinus* (isolate PL1) has also shown all type 1, 2 and 3 effects on *T. saginata* eggs throughout the studied intervals. However, for type 3 effect this fungus had 23.8%, 25.4% and 24.8% in intervals 5, 10 and 15 days, respectively.

Through light microscopy, 40x objective, *T. saginata* eggs were observed, destroyed by fungi *P. chlamydosporia* and *P. lilacinus*, characterizing its ovicidal activity (type 3 effect).

DISCUSSION

According to Lysek et al[18], the main characteristic of an ovicidal fungus is to have type 3 effect during the infection process of eggs. In this work, this effect was demonstrated by isolates VC1, VC4 and PL1 on *T. saginata* eggs.

Table 1. Percentages and standard deviations of the ovicidal activity (effects of type 1, 2 and 3) of the nematophagous fungi *Pochonia chlamydosporia* (VC1 and VC4), *Paecilomyces lilacinus* (PL1) and the control group without fungi on *Taenia saginata* eggs in the intervals of 5, 10 and 15 days of interaction

	Effect at 5 days		
Isolates	**Effect at Type 1***	**Effect at Type 2****	**Effect at Type 3*****
VC1	$23.9^{A} \pm 6.3$	$21.7^{A} \pm 17.9$	$12.8^{A} \pm 11.6$
VC4	$18.4^{A} \pm 6.0$	$9.9^{B} \pm 5.4$	$2.3^{B} \pm 1.9$
PL1	$24.8^{A} \pm 10.8$	$26.5^{A} \pm 9.5$	$23.8^{C} \pm 11.8$
Control	$0^{B} \pm 0$	$0^{C} \pm 0$	$0^{D} \pm 0$
	Effect at 10 days		
Isolates	**Effect at Type 1***	**Effect at Type 2****	**Effect at Type 3*****
VC1	$24.0^{A} \pm 8.3$	$24.2^{A} \pm 8.5$	$18.2^{A} \pm 9.3$
VC4	$17.5^{A} \pm 4.9$	$12.3^{B} \pm 5.4$	$7.0^{B} \pm 3.8$
PL1	$24.2^{A} \pm 7.8$	$25.5^{A} \pm 7.8$	$25.4^{A} \pm 8.1$
Control	$0^{B} \pm 0$	$0^{C} \pm 0$	$0^{C} \pm 0$
	Effect at 15 days		
Isolates	**Effect at Type 1***	**Effect at Type 2****	**Effect at Type 3*****
VC1	$22.3^{A} \pm 8.4$	$22.2^{A} \pm 8.4$	$9.7^{A} \pm 5.7$
VC4	$17.2^{A} \pm 5.7$	$17.2^{B} \pm 6.3$	$8.0^{A} \pm 4.0$
PL1	$25.3^{A} \pm 11.2$	$29.0^{A} \pm 8.7$	$24.8^{B} \pm 8.0$
Control	$0^{B} \pm 0$	$0^{C} \pm 0$	$0^{C} \pm 0$

Percentages followed by the same capital letter in the same column do not present any statistical differences (P>0.05) – Friedman test. Physiological and biochemical *effect, without morphological damage to the egg shell, where the hyphae are observed adhered to the shell. **effect lithic with morphological alteration of the egg shell and the embryo, without the penetration of the hyphae through the shell. *** effect Lithic with morphological alteration of the shell and the embryo, besides the penetration of hyphae and egg internal colonization.

No difference (P>0.05) between VC1 and VC4 for type 1 effect was observed. For type 2 effect, the action of isolate VC1 was higher than isolate VC4, with statistical difference (P<0.05) in all time intervals noticed. For type 3 effect, on 5th and 10th days of interaction, difference (P<0.05) in the ovicidal activity between VC1 and VC4 was noticed, where the action of isolate VC1 was higher. However, it is discussed that the action of fungal isolates (VC1 and VC4) against intestinal helminth eggs has not shown differences (p> 0.01)[20]. Therefore, the use of both *P. chlamydosporia* isolates in the biological control of gastrointestinal helminth eggs can be suggested.

Literature reports that, even though there is difference in the ovicidal activity, both isolates VC1 and VC4 could be successfully used in the destruction of eggs

of several genus of gastrointestinal helminth, since they have type 3 effect. Araujo et al[9] recorded difference for the ovicidal activity between isolates VC1 and VC4, but showing the destruction of *T. saginata* eggs parasitized at the end of the experimental test. In the other hand, Braga et al[11] assessed the efficacy of isolates VC1 and VC4 on *Fasciola hepatica* eggs and did not observed difference (P> 0.01) in percentage results for the type 3 effect between them. Similarly, these authors in another work, assessing the action of these isolates over *Schistosoma mansoni* eggs, did not record any difference (P>0.01) for the type 3 effect throughout the studied intervals[12].

On the other hand, *P. chlamydosporia* has other characteristics required for used as biological control agents of domestic animal gastrointestinal helminthiasis. The production of chlamydospores, for instance, which are resistant structures that enhances the fungus survival in the soil and helps its passage through the gastrointestinal tract of domestic animals. In addition, this fungus is harmless to animals, humans and the environment[11].

This information is interesting because it determines that fungus *P. chlamydosporia* is promising.

Regarding time of interaction of fungus over parasitized eggs (5-, 10- and 15-day intervals) in the present work, it was noted that the ovicidal action of VC1 and VC4 is similar to the results presented in other works[11, 12]. These authors have shown the efficacy of VC1 and VC4 over *F. hepatica* and *S. mansoni* eggs at the end of 21 days. This demonstrates that this fungus has efficacy in short time window.

Fungus *P. lilacinus* was compared with two fungal isolates of the species *P. chlamydosporia* (VC1 and VC4). When compared to type 1 effect, both do not have statistical difference (P>0.05), but when compared regarding type 2 effect, the isolate *P. lilacinus* had difference (P<0.05) from isolate VC4 in all observed days, where *P. lilacinus* had greater efficacy, unlike what was assessed with isolate VC1, which had no statistical difference (P>0.05) for isolate *P. lilacinus*. Regarding type 3 effect, fungus *P. lilacinus* had difference (P<0.05) for isolate VC1 at 5 and 15 days, not being noticed difference in the 10th reading day. For isolate VC4, fungus *P. lilacinus* had statistical difference (P<0.05) throughout all reading days.

However, all isolates were efficient on destroying *T. saginata* eggs. The action of *P. lilacinus* as potential biological controller of cestodas eggs has been mentioned in other works[14, 15]. On the other hand, this fungus is also effective on destroying nematodes eggs[17].

T. saginata eggs are resistant in the environment, to conventional sewage treatment and remains viable for up to 12 months, since they have a protective shell called embryophor[21]. In this way, alternative measures that can be employed to fight the environmental dissemination of this and other gastrointestinal parasites and their infective forms are important. Among them, biological control by ovicidal fungi that spread in the fecal environment[15].

On the other hand, the penetration mechanism of ovicidal fungi in parasitized eggs is not yet fully clear. However, it is acknowledge that physical and mechanical activity, followed by enzymatic activity is one of the main compounds in the process and attack and penetration through eggs. In addition, there is the need of comparison of ovicidal activity of ovicidal nematophagous fungi on eggs of gastrointestinal helminth parasites[9].

CONCLUSION

In this work, the efficacy in action of fungi *P. chlamydosporia* and *P. lilacinus* in destroying *T. saginata* eggs was evidenced. Although there was statistical difference among isolates, all of them can be used in biological control of eggs of this cestoda.

Acknowledgements

The authors thank FAPEMIG, CAPES, FINEP Pró-equipamentos and CNPq for financial support.

REFERENCES

1. Jongwutiwes S, Putaporntip C, Chantachum N, Sampatanukul P. Jejunal perforation caused by morphologically abnormal *Taenia Saginata* infection. J Infect. 2004; 49:324-8.
2. Lateef M, Zagar SA, Khan AR, Nazir M, Shoukat A. Successful treatment of niclosamide- and praziquantel-resistant beef tapeworm infection with nitazoxanide. Int J Infect Dis. 2008; 12:80-2.
3. Pereira MAVC, Schwanz VS, Barbosa CG. Prevalência da cisticercose em carcaças de bovinos abatidos em matadouros-frigoríficos do estado de Rio de Janeiro, submetidos ao Controle do Serviço de Inspeção Federal (SIF-RJ), no período de 1997 a 2003. Arq Inst Biol. 2006; 73:83-7.
4. Acha P, Szifre B. Zoonosis y enfermidades trasmissible comunes al hombre y a los animales. p. 989, 2006.

5. Gemmell MA, Lawson JR. Ovine cysticercosis: an epidemiological model for the cysticercosis. I. Free-living egg fase. In: Flisser A. Cysticercosis: present stage of knowledge and perspectives. New Cork. 1986;87-98.
6. Carrada-Bravo T. Teniasis-cisticecosis como problema de salud pública. Boletín Médico del Hospital Infantil del México. 1987; 44:427-34.
7. Rey L. Bases da Parasitologia Médica. Guanabara Koogan, Rio de Janeiro; 2008.
8. Organización Panamericana de La Salud. Epidemiologia y control de la teniasis/cisticercosis en America Latina: 1994, Washington.
9. Araujo JM, Araújo JV, Braga FR, Carvalho RO, Silva AR, Campos AK. Interaction and ovicidal activity of nematophagous fungus *Pochonia chlamydosporia* on *Taenia saginata* eggs. Experiment Parasitol. 2009; 121:338-41.
10. Araújo JV, Mota MA, Campos AK. Controle biológico de helmintos parasitos de animais por fungos nematófagos. Rev Bras Parasitol Vet. 2004; 13:165-70.
11. Braga FR, Araújo JV, Campos AK, Araujo JM, Carvalho RO, Silva AR et al. *In vitro* evaluation of the effect of the nematophagous fungi *Duddingtonia flagrans*, *Monacrosporium sinense* and *Pochonia chlamydosporia* on *Fasciola hepatica* eggs. World J Microbiol Biotechnol. 2008a; 24:1559-64.
12. Braga FR, Araújo JV, Campos AK, Araujo JM, Silva AR, Carvalho RO et al. *In vitro* evaluation of the effect of the nematophagous fungi *Duddingtonia flagrans*, *Monacrosporium sinense* and *Pochonia chlamydosporia* on *Schistosoma* mansoni eggs. World J Microbiol Biotechnol. 2008b; 24:2713-16.
13. Araújo JV, Braga FR, Araujo JM, Silva AR, Tavela AO. *In vitro* evaluation of the effect of the nematophagous fungi *Duddingtonia flagrans*, *Monacrosporium sinense* and *Pochonia chlamydosporia* on *Ascaris suum* eggs. Parasitol Res. 2008; 102:787-90.
14. Braga FR, Araújo JV, Araujo JM, Carvalho RO, Silva AR. Efeito do Fungo *Paecilomyces lilacinus* sobre ovos de *Taenia saginata*. Rev Soc Bras Med Trop. 2008c; 41:686-8.
15. Braga FR, Araújo JV, Carvalho RO, Silva AR, Araujo JM, Tavela AO et al. Ovicidal effect of nematophagous fungi on *Taenia taeniaeformis* eggs. World J Microbiol Biotechnol. 2008d; 25: 533-5.
16. Urquhart GM, Armour J, Duncan JL, Dunn AM, Jennings FW. Parasitologia Veterinária, 2nd ed. Guanabara Koogan, Rio de Janeiro: 1998.
17. Araújo JV, Santos MA, Ferraz S. Efeito ovicida de fungos nematófagos sobre ovos embrionados de *Toxocara canis*. Arq Bras Med Vet Zootec. 1995; 47:37-42.
18. Lysek H, Fassatiová O, Pineda NC, Hernández NL. Ovicidal fungi in soils of Cuba. Folia Parasitol. 1982; 29:265-70.
19. Ayres M, Ayres JRM, Ayres DL, Santos AS. Aplicações estatísticas nas áreas de ciências biológicas. 290pp. Brasília, 2003.
20. Braga FR, Ferreira SR, Araújo JV, Araujo JM, Silva AR, Carvalho RO et al. Predatory activity of *Pochonia chlamydosporia* fungus on *Toxocara* (syn. *Neoascaris*) *vitulorum* eggs. Tropic Animal Health Product. 2009; 42:309-14.
21. Silva AVM. Teníase e Cisticercose. In Neves DP, Melo AL, Linardi PM. Parasitologia Humana. 11 ed. Atheneu: São Paulo; 2005.