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# *In vitro* predatory activity of *Arthrobotrys oligospora* and after passing through gastrointestinal tract of small ruminants on infective larvae of trichostrongylides

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ABSTRACT

*In vitro* predatory activity of 157 native isolates of *Arthrobotrys oligospora* from China on larvae of trichostrongylides (*Trichostrongylus colubriformis* and *Haemonchus contortus*) in feces of sheep were assessed. The results showed that 135 of tested isolates of *A. oligospora* reduced the development of trichostrongylide larvae in feces by 90–99.99%, 11 isolates by 80–89.46% and 11 isolates by 14.58–78.82%. To understand their capacity of passing through gastrointestinal tract of sheep, 50 native isolates of *A. oligospora* were selected and assessed in sheep. Among these isolates, 16 isolates significantly reduced the number of larvae developing in the feces ($P < 0.05$); their percentage reduction of L3 ranged from 42.87% to 99.51% and the isolates tested were harvested in 5 g sub-samples of from sheep in each treatment group, indicating that these isolates had the capacity of preying larvae of trichostrongylides after the passage through gastrointestinal tract of sheep. The remaining isolates of *A. oligospora* were not able to survive after passage through gastrointestinal tract of sheep. In the following, the 16 isolates that presented more or less viability after sheep gastrointestinal passage were selected and assessed in goats. The results showed that the 11 isolates out of them could be able to pass through the digestive tract of goats without loss of ability of preying larvae of trichostrongylides in feces and their efficacies ranged from 53.88% to 94.28%, and that the isolates tested were harvested in 5 g sub-samples of feces from goats in each treatment group. In the current study, these isolates which demonstrated outstanding properties *in vitro* and could survive in the passage through the alimentary tract of sheep and goat should be potential candidates as a possible feed additive.



## 1. Introduction

Gastrointestinal nematode (GIN) infection is a serious problem for domestic ruminants under the grazing system. These parasites in small ruminants mainly include *Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus* spp., *Nematodirus* spp., *Marshallagia* spp., *Chabertia* spp., and *Cooperia* spp. in northwest China (Cai and Bai, 2009), which causes diseases and can kill ruminants. However, the greatest economic impact is in the growth decrease of young animals, resulting in low productivity. The traditional method of nematode control has been the use of anthelmintics, which also provoked the selection of helminthes populations that are resistant to the different chemical groups. To date, considerable anthelmintic routine (e.g., benzimidazole, levamisole and ivermectin) has lost or reduced their efficacy due to the resistance developed by GIN (Falzon et al., 2013; Menkir et al.,

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2006; Whittaker et al., 2016). The rapidly escalating worldwide problem of anthelmintic resistance in parasitic nematodes of ruminants and the increasing concern with chemical residues in livestock products and the environment have resulted in the search for other control methods.

In order to decrease in frequency of treatments with anthelmintic, the integration of other methods of parasite control that aim at decreasing the number of infective larvae in pastures and providing the decrease of parasite load in animals is welcomed (Braga et al., 2009a,b; Araújo et al., 2010). Among the alternative measurements for chemotherapy, the application of biological control by nematophagous fungi has presented promising results *in vitro* and *in vivo* (Braga and Araújo, 2014; Larsen, 1999). In the nematophagous fungal groups, *Duddingtonia flagrans* as a biocontrol tool after passage through the gastrointestinal tract of domestic animals can effectively reduce infective larvae in the feces of sheep (Sagüés et al., 2011; Silva et al., 2010), donkeys (Araújo et al., 2012), goats, cattle, horses, dogs, and pigs (Braga and Araújo, 2014). Other nematophagous fungi, such as *Arthrobotrys thaumasia* (*Monacrosporium thaumasium*), *Arthrobotrys (Monacrosporium) sinense* and *Arthrobotrys robusta*, have been evaluated for predatory activity against larvae of gastrointestinal helminthes and ability for passing through gastrointestinal tract of domestic animals (Braga et al., 2009a,b; Tavela et al., 2012). *Arthrobotrys oligospora*, the first recognized nematode-trapping fungus by Zopf at 1888, is the most commonly isolated all over the world and was more frequently occurring in various habitats and different substrates, including almost all types of natural soil, animal feces, coniferous leaf litter and *etc*. (Niu and Zhang, 2011).

In the aspect of biological control of parasitic nematodes in animals, many laboratory experiments have evaluated the *in vitro* ability of *A. oligospora* to destroy infective larvae of parasitic nematodes in domestic animal and investigated the effect of physical factors and medium composition on trap formation of the fungus (Bird and Herd, 1995; Chandrawathani et al., 1998; Scholler and Rubner, 1994; Sissay et al., 2006; Waller et al., 1994; Migunova and Byzov, 2005). In addition, some field trials have been conducted for attempts to control infection with *Ostertagia ostertagi* and *Cooperia oncophora* (Trichostrongylidae) in grazing calves by adding mycelium of the nematode-trapping fungus *A. oligospora* to cow pats (Grønvold et al., 1987, 1988, 1989). However, adding the nematophagous fungi to the feedstuff or direct eating is the developmental trend for the biological control preparation of parasitic nematodes in animals. After the feedstuff passing through the digestive tract, the fungi are excreted with the feces and eggs. Under the appropriate temperature and humidity, the spores in the feces germinated to mycelia, which differentiated to predatory structures for capturing larvae and leaded to reduction of the infective larvae in feces. For *A. oligospora,* some studies have tested the survival and performance of stress selected after passage through the gastrointestinal tract of domestic animals (Grønvold et al., 1993; Sanyal et al., 2009; Wang et al., 2014). These results are not consistent, and most of the results showed that the conidia of *A. oligospora* lost its ability to survive after passing the animal's digestive tract.

It should be project of future research to screen more of the potential candidates for the biological control of parasitic nematodes from the native isolates of nematophagous fungi. As there are differences in the performance of the fungal isolates from different regions, native isolates are more adapted to local climate and geographical conditions. If alien nematophagous fungi are introduced, there may also be a risk of alien species invasion and gene recombination. For that consideration, from October 2012 to October 2014, we isolated 288 isolates of *A. oligospora* from 1502 samples associated with sheep and cattle (Cai et al., 2016). The objective of the present study was to deal with screening the isolates of *A. oligospora* of the predacious fungus isolated from the samples associated with sheep and cattle in China by *in vitro* and *in vivo* tests for their suitability as biological control agents.

## 2. Materials and methods

### 2.1. In vitro experiment of fungal isolates

157 Chinese isolates of *A. oligospora* were kept in sealed assay tubes containing 2% corn meal agar (CMA) at 4 °C and in the dark. Fungal mycelia and conidia from the assay tubes were inoculated on 2% water agar (WA) plates for 7 days in order to test the viability and purity. One piece of inoculum (approximately 5 mm in diameter) from the 2% WA plate was transferred to 250 mL Erlenmeyer flasks with 150 mL liquid potato-dextrose medium (Difco), pH 6.5 and incubated under agitation (160 rpm) in the dark at 26 °C for 7 days. After this period, the mycelia were extracted, filtered, and then weighed using an analytic balance. Then the mass of the mycelium was diluted with sterile distilled water by 1:5, dispersed by vortexing and ready to use.

The experiment was conducted at core facility of experimental animal in Yuzhong campus of the Northwest University for nationalities, Lanzhou, China, latitude 35 ° 47′18.4″N, longitude 104 °09′7.5″E.

Eighteen crossbred male Small Tail Han lambs (three to four month old, live weight 18.4–22.7 kg, ave. 19.3 kg) were kept in a stalls and were drenched at twice with a combination of albendazole/levamisole anthelmintics (15 and 7.5 mg/kg liveweight, respectively) according to the manufacturer's recommended dose to remove existing worm burdens. The animals were moved indoors and received fresh water *ad libitum* and were daily fed with lucerne hay and balanced ration for sheep. 7 days after the anthelmintic treatment, all animals were infected with 10,000 L3 of trichostrongylides (composed of approximately 40% *H. contortus* and 60% *T. colubriformis*) (Wang et al., 2015), which were administered orally by syringe, in a small volume of water, as two equal doses on consecutive days. After the parasite infections was patent (approximately at 3 weeks after infection), fecal samples were collected for fecal nematode egg count (FEC) and for each sample, FEC was estimated using a modified McMaster method in which 1 egg counted equates to 50 eggs/g (epg) in order to understand the degree of nematode infection in each infected sheep.

Feces of animals were collected using a collecting bag composed of cotton cloth. The technique of Waghorn et al., (2003) was modified for coprological culture. Each culture consisted of 10 (±0.1) g of and 2 mL fungal suspension that were mixed with toothpick placed in the base of a 5.5 cm diameter Petri dish, in which a small amount of sterile saw dust and distilled water were added to adjust humidity and ventilation when necessary. The Petri dish was then placed inside a 9 cm diameter tissue culture dish. Approximately 10 mL of distilled water was added to the base of the tissue culture dish to maintain humidity and prevent escape of larvae. The lid of the tissue culture dish with its unique identifying label was then put on and the culture incubated for 12 days at 25 °C. The water in the tissue culture dish was checked regularly over the duration of the culture period and replenished if required. Three parellel repeats were performed for each fungal isolates, while control groups using 2 mL distilled water instead of fungal isolates were performed under the same condition in three parellel repeats as well. Following incubation, the active L3s were extracted from the fecal cultures and concentrated into a hemolysis tube by a modified Baermann apparatus (Bar-Scante et al., 2003), with water at 42–45 °C and waiting 24 h for decantation. The total nematodes in the tube were directly estimated by counting. Except for the tube,