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Test the Efficacy of a *Arthrobotrys oligospora* N Mutant in Nematode-Trapping Larvae after Passage through the DigestiveTract of Sheep

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Abstract: For oral application biocontrol of animal parasitic nematode in ruminants, ion beam implantation nematode-trapping fungi must has the capacity to survive the passage through the digestive tract and be efficient in reducing infective larvae of nematodes in the faeces. Ion beam induced mutation the spores of *Arthrobotrys oligospora* of nematode-trapping. The mutant of the genetic stability is breeded. The fungi were cultured in bottles with corn kernels as a growth media and spores of different doses was respectively administered orally to each group of sheep naturally infected with gastrointestinal nematodes. The control group did not receive fungi. The faeces of these experimental animals were colleted and fecal cultivations carried out. Tested the fungal germination, growth, reproduction and predation livestock parasitic nematode larvae in laboratory. Test the efficacy of a *Arthrobotrys oligospora* N mutant in nematode-trapping larvae after passage through the digestive tract of sheep. In this research, these capacities were evaluated. The results indicated that ion beam implantation nematode-trapping fungi is a positive mutation. Mutant spores through the digestive tract of sheep can kill livestock parasite nematode larvae *in vitro*. These results indicate the potential of *Arthrobotrys oligospora* N mutant as a biological control agent for sheep nematodes. This study showed that such biotechnology can be explored for improving the effectiveness of the use of fungal infections to control livestock parasitic nematodes. This research represents the first application of nematode-trapping fungi in eukaryotic microorganisms.

Key words: Nematode-trapping fungi, *Arthrobotrys oligospora* N, ion beam, digestive tract, China

INTRODUCTION

With the drawbacks of the a variety of the chemical drug-worming medicine been continuously awareness, biological control using the predation nematode fungi has attracted much interest as an alternative to chemical methods of controlling livestock parasite nematode (Larsen *et al*., 1994, 1995). Prevention and treatment the livestock parasitic nematode disease using the predation nematode fungi are carried out, the development of non-polluting livestock products has an extremely important significance. The production spore of the nematode-trapping fungi isolated from the nature limit the application of biological control of livestock parasites in a broad range. Nematode-trapping fungi can be breeded by the ion beam mutation (Li *et al*., 2011, 2012; Song *et al*., 1998, 1999; Wu *et al*., 2005; Xu *et al*., 2010; Yu *et al*., 1991). The spread of the nematodes of livestock parasites is larvae hatched by eggs in livestock *in vitro*. The nematode-trapping fungi can kill livestock parasitic nematode larvae. It is the key whether nematode-trapping fungi mutagenesis spores can resist the livestock digestive tract, to catch and feed on parasitic nematode larvae *in* livestock *vitro*. Test a *Arthrobotrys oligospora* N mutant strains through sheep gastrointestinal. Establish foundation for the use of nematode-trapping fungi prevention against parasitic nematodes of livestock.

MATERIALS AND METHODS

Test biological and equipment: *A. oligospora* N and experimental animals by the Inner Mongolia Agricultural University College of Veterinary Medicine to provide. In accordance with the standard method, prepared 0.4 g L^{-1} corn meal agar, conidia corn grain culture medium. Implantation the ion beam device, produced by the Hefei Institute of Plasma Physics, Chinese Academy of Sciences.

Strongylus equines larvae, take the horse fresh stool and place on the enamel tray (20×40 cm) were cultured at 25°C for 15 days. Separated and collected the 3rd stage larvae by the modified Baermann's method and purificated. Under an optical microscope examination, >95% of the larvae maintain their vitality. Count after the

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3rd stage larvae of the strongylus equines, suspension was diluted to 300 worm mL^{-1} concentration ready to use.

Preparation of the starting test strain: The strains of *A. oligospora* were inoculated at 0.4 g L^{-1} corn meal agar medium, cultured at 25±1°C incubator for 3 weeks, added spores eluent, stirred with a sterile glass rod, eluted conidia in a small amount of multiple, collected spores eluent and placed in 230 rpm shaker flask containing glass beads to break up for 25 min, spores are shaked to break up with a sterile gauze filtered twice and then in the oscillator oscillation blended, made of 10^6~10^7 single spore mL^{-1} conidia suspensions. Draw the 0.1 mL spore suspension coating on the sterile empty Petri dish surface, air-dried made spores membrane in the clean bench for subsequent ion beam implantation.

Ion beam implantation: Ion N^+ the energy is 10 keV, the target chamber vacuum is 10^{-3} Pa dose of an $130\times2.6\times10^{13}$ N^+ ions cm^{-2} nitrogen ion beam induced mutation conidia of *A. oligospora*. Two control design: A dry control also known as blank control, the other for vacuum control. The residence time of spores of different implantation doses in the target chamber is the same. Each group is three Petri dishes in control and experimental group.

Mutagenicity spores by ion beam in increments of 10^{-1}-fold dilution is cultured. Each dilution were inoculated in the three media. Breeding of the mutation fungal of *A. oligospora* N culture. Mycelium and single spore are selected breeding mutant fungal strains. Selected *A. oligospora* N and cultured for 10 generations, the genetic stability test.

Batch culture of the mutagenicity fungal spores of *A. oligospora* N: The strains isolated single conidial were inoculated in the preparation of a conidial corn grain medium. After cultured for 3 weeks, until conidia produced a lot.

Animal experiments: Total 16 test sheep were divided into A-D, 4 groups, group A is the control group; B, C and D group are the test group, the average sheep weighing is approximately 50 kg. About 15 g of fresh feces of test animals were collected before the test, placed into the Petri dish, cultured at 20±1°C incubator, flip, add water daily. In accordance with the relevant information (Foreyt and Foreyt, 2002), separated and observed the 3rd stage larvae for 15 days. It is determinated what is the animal parasitic worms species. This is preliminary.

Corn kernels of different spores doses of *A. oligospora* N was respectively administered orally to each group of sheep naturally infected with gastrointestinal nematodes. The experimental animals are allowed *ad libitum* clean. About 15 g corn kernels contained 6, 9, 12×10^7 spores of *A. oligospora* N were fed experimental animals. Then, respectively, 48 h after feeding fungi spores, taked feces on interval of 12 h. The faeces of these animals were colleted for 3 days pre- and 3 days post-administration of the corn kernels and fecal cultivations carried out for 10 days, separated larvae by the Baermann's method, recorded statistics of the number of grams of fecal larvae. Compared the control group and experimental group the number of killing larvae rates.

After 48 h, test samples were collected. About 1 g of fresh feces of experimental animals inoculated at 0.4 g L^{-1} corn meal agar. The faeces of each animal inoculated with 3 Petri dishes, set 20±1°C incubator to cultivate when the mycelium covered on petri dishes, the culture dish by adding live nematode 3rd stage larvae continue to placed in the incubator, culture, observed with or without predator (Fungi ring, kungi network) generation and the presence of capturing parasites larvae.

RESULTS

A dose of mutagenesis spores is analyzed from early germination, spores volume, predation larval rate. The results of this experiment have mutagenic *A. oligospora* effect. The spores of *A. oligospora* were induced mutation to become a more good strain in development, reproduction, the amount of production of spores, predation larval. Genetic traits of breeded *A. oligospora* N are stable for 10 generations cultured.

The 3rd stage larvae of parasitic nematode of examination in the stool of these animals before the test is as follows: *Oesophagostomum*, *Trichostrongylus*, *Oestertagia*, *Nematodirus* and *Chabertia*.

Different dose group, the experimental group B-D, the insecticidal rate were 95.3, 95.8 and 94.6%. Experimental group C, 15 g corn kernels contained spores 9×10^7 is the highest insecticide rate.

Corn kernels containing conidia of the *A. oligospora* N were administered orally to experimental animals, 48 h after. The feces collected experimental animals were cultured, the ability to test that the *A. oligospora* N prey the 3rd stage larvae showed that mutations spores can be through the sheep digestive trac. Volume hurled the sheep *A. oligospora* N spores is insecticidal the highest rate in 9×10^7 spores/sheep (Table 1).

After 48 h test, experimental animal droppings in the 0.4 g L^{-1} corn meal agar medium were cultured. There are *A. oligospora* N spores and hyphae in the meal agar medium. Fungi ring and network were produced in medium

Table 1: The killing efficiency of *A. oligosporaon* larvae

Project	Spores/sheep	Average kill worm (%)
Control group A	0	0.00
Experimental group B	6×10^7	94.8
Experimental group C	9×10^7	96.4
Experimental group D	12×10^7	95.2

joined livestock parasite nematode larvae. Livestock parasites larvae in the culture medium were captured. It is proved that hurled conidia passed through the experimental animal digestive tract and survived in the stool, developed and preserved the activity of its prey.

DISCUSSION

The use of biological control methods control livestock parasitic worm disease, natural enemies of nematode-trapping fungi is very potential. The ideal of nematode-trapping fungi is growth rate fast, predation rate high, producing large amount of spores of the strains. $130\times2.6\times10^{13}$ N^+ ions cm^{-2} ion beam implantation spores of *Arthrobotrys oligospora* able to get a more good *A. oligospora* N. Conidia of *A. oligospora* N through the digestive tract of the experimental animals can still survive. Spores of *A. oligospora* N excreted with dung, the fungi in the feces carry on development, growth, producing hyphae, reproduction, production of spores, producing a predator (fungi ring, fungi network) by the nematode larvae stimulation, capturing and degradation nematode larvae. The nematode larvae is lethal. Biological control of livestock parasitic nematode in livestock fed spores of *A. oligospora* N is feasible. Lay a good foundation for the clinical application of nematode-trapping fungi. The corn kernels contained *A. oligospora* N spores was administered orally to livestock, control of animal parasitic nematode is the first time. Low-energy nitrogen ion beam implantation of nematode-trapping fungi is the first time in the world.

There had been some reported research that parasitic nematode larvae is predated by nematode-trapping fungal (Gronvold *et al*., 1985). Test is to use the 3rd stage larvae cultured in the culture medium (Gronvold *et al*., 1985). This experiment research the 3rd stage larvae of natural infection under laboratory conditions, compared with the control group, test group the number of nematode larvae to reduce by 94.8-96.4%. Only feeding of *Arthrobotrys oligospora* N significantly reduced the percentage of the 3rd stage larvae in the fecal cultivations.

It is the best that *A. oligospora* N spores are fed to livestock in the clinical application. Parasitic nematodes eggs and the spores in the manure of livestock will be discharged together. *A. oligospora* N play to kill the parasite nematode larvae. The results of cultured the mutagenesis spores in this experiment confirmed that the spores through animal digestive tract can still survive. The spores of *A. oligospora* N grow, develop, produce hyphae in the stool and *A. oligospora* N developed predator control by the stimulation of the hatching of nematode larvae. The nematode larvae was captured to death. These results indicate the potential of *Arthrobotrys oligospora* N as a biological control agent for sheep nematodes.

Data shows very clear by Larsen *et al.* (1997) and Krecek and Guthrie (1999). Application for the nematode-trapping fungi strains isolated under the conditions of the local natural environment is very important to the local livestock parasitic nematode control because the same nematode-trapping fungi in indifferent regions may be the existence of strain differences (Wang *et al*., 2010). The only fungi separation from the local strains can be used for the prevention of parasitic nematodes of livestock. There have not been reports that corn kernels contained conidia control animal parasitic nematode in clinical trial studies. Corn kernels contained conidia in batch culture of the strains of this experiment were done clinical trials killing parasitic nematode larvae and achieved good results.

CONCLUSION

Ion N^+ the energy is 10 keV, the target chamber vacuum is 10^{-3} Pa dose of an $130\times2.6\times10^{13}$ N^+ ions cm^{-2} nitrogen ion beam induced mutation conidia of *A. oligospora*. Mutagenic fungal spores can resist the role of the sheep digestive tract. About 15 g corn kernels containing 9×10^7 spores of *A. oligospora* N can kill livestock parasite nematode larvae. The ion beam implantation nematode-trapping fungi spores can be bred mutant fungal strains useful in practical clinical biological control of livestock parasites nematode.

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