Kafkas Univ Vet Fak Derg
25 (4): 435-444, 2019
DOI: 10.9775/kvfd.2018.20603

Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

Enzymological Properties and Nematode-Degrading Activity of Recombinant Chitinase AO-379 of *Arthrobotrys oligospora*

Wenqiang ZHONG [1,†] Ying CHEN [2,†] Shasha GONG [1,†] Jun QIAO [1,3] Qingling MENG [1]
Xingxing ZHANG [3] Xifeng WANG [1] Yunfu HUANG [1] Lulu TIAN [1] Yanbing NIU [2]

[†] *These authors contribute equally*

[1] Key Laboratory of Control and Prevention of Animal Disease of Xinjiang Production & Construction Corps, College of Animal Science and Technology, Shihezi University, Shihezi, Xinjiang, 832003, CHINA

[2] College of Animal Science and Technology, Xinjiang Agricultural Vocational and Technical College, Changji, Xinjiang 31100, CHINA

[3] State Key Laboratory for Sheep Genetic Improvement and Healthy Production, Xinjiang Academy of Agricultural and Reclamation Science, Shihezi, Xinjiang, 832000, CHINA

Article ID: KVFD-2018-20603 Received: 25.07.2018 Accepted: 20.03.2019 Published Online: 24.03.2019

How to Cite This Article

Zhong W, Chen Y, Gong S, Qiao J, Meng Q, Zhang X, Wang X, Huang Y, Tian L, Niu Y: Enzymological properties and nematode-degrading activity of recombinant chitinase AO-379 of *Arthrobotrys oligospora*. *Kafkas Univ Vet Fak Derg,* 25 (4): 435-444, 2019. DOI: 10.9775/kvfd.2018.20603

Abstract

Chitinase is an important virulence factor produced by nematode trapping fungi in the process of infection, and plays an important role in the cleavage of nematodes and their eggshells. In this study, the cDNA sequence of *Arthrobotrys oligospora* chitinase AO-379 was amplified by RT-PCR and inserted into the vector pPIC9K to induce the expression of AO-379 in *Pichia pastoris* GS115. The recombinant AO-379 (reAO-379) was purified by nickel ion affinity chromatography, and enzymological properties and nematode-degrading activity of reAO-379 was analyzed. SDS-PAGE and Western blot analysis showed that the reAO-379 with molecular weight of about 44 kDa was successfully obtained. The reAO-379 showed strong chitinase activity at pH 5.5 and 30°C. Using reAO-379 to treat *Strongylus equinus, Caenorhabditis elegans* and *Haemonchus contortus* for 12, 24, and 36 h , the killing rates of reAO-379 in *S. equinus* were 42%, 89% and 100%; in *C. elegans* were 50%, 90% and 97%; in and H. contortus were 53%, 62% and 84%, respectively. Using reA-379 to treat *Fasciola hepatica* and *Dicrocoelium chinensis* eggs for 24, 48 and 72 h, the degradation rates of reAO-379 were 12%, 43% and 65% in F. hepatica eggs, and were 15%, 33% and 55% in *D. chinensis* eggs, respectively. Our study suggests that the reAO-379 is potentially valuable for development of biological control agent against digestive tract nematodes in livestocks.

Keywords: *Chitinase AO-379, Molecular characteristics, Expression, Nematode-degrading activity, Arthrobotrys oligospora*

Arthrobotrys oligospora Rekombinant Çitinaz AO-379'un Enzim Özellikleri ve Nematod İndirgeyici Aktivitesi

Öz

Çitinaz, enfeksiyon sürecinde nematod tutucu mantar tarafından üretilen önemli bir virulans faktörüdür ve nematodların yumurta kabuklarından ayrılmalarında önemli rol oynar. Bu çalışmada, *Arthrobotrys oligospora* çitinaz AO-379 cDNA sekansı RT-PCR ile amplifiye edildi ve Pichia pastoris GS115'de AO-379 ekspresyonunu oluşturmak amacıyla pPIC9K vektörüne yerleştirildi. Rekombinant AO-379 (reAO-379) nikel iyon affinite kromotografi ile saflaştırıldı, enzim özellikleri ve nematod indirgeme aktivitesi araştırıldı. SDS-PAGE ve Western blot analizleri yaklaşık 44 kDa moleküler ağırlığında olan reAO-379'ın başarıyla elde edildiğini gösterdi. reAO-379 pH 5.5 ve 30°C'de güçlü çitinaz aktivitesi gösterdi. reAO-379 ile *Strongylus equinus, Caenorhabditis elegans* ve *Haemonchus contortus* 12, 24 ve 36 saat muamele edildiklerinde sırasıyla *S. equinus* için %42, %89 ve %100, *C. elegans* için %50, %90 ve %97 ve *H. contortus* için %53, %62 ve %84 öldürme oranlarına sahip olduğu gözlemlendi. *Fasciola hepatica* ve *Dicrocoelium chinensis* yumurtaları reA-379 ile 24, 48 ve 72 saat muamele edildiğinde, indirgenme oranları *F. hepatica* yumurtalarında sırasıyla %12, %43 ve %65 iken *D. chinensis* yumurtalarında sırasıyla %15, %33 ve %55 olarak belirlendi. Bu çalışma, reAO-379'un yetiştiricilikte sindirim sistemi nematodlarına karşı potansiyel bir biyolojik kontrol ajan olarak kullanılabileceğini göstermiştir.

Anahtar sözcükler: *Çitinaz AO-379, Moleküler özellikler, Ekspresyon, Nematod-indirgeyici aktivite, Arthrobotrys oligospora*

INTRODUCTION

Animal digestive tract parasitic nematodes are a class of parasites that cause serious damage to livestock husbandry, leading to huge economic losses to the world's livestock industry each year [1]. In recent decades, the use

İletişim (Correspondence)
+90 86-993-2055036 Fax: 86-993-2055038
xjmqlqj@163.com

of chemical anthelmintic drugs, to a large extent, has relieved nematode caused economic losses in livestock husbandry. However, long-term, high-dose chemical drugs also cause serious problems such as drug resistance of nematodes, drug residues in food products of animal origin, destruction of ecosystems and biodiversity, and pollution to soil and water sources [2,3]. Therefore, there is an urgent need to develop effective animal and environment-friendly biological agents to control digestive tract nematodes in livestocks [4].

Nematode trapping fungi are one of the natural predators of nematodes, which can capture nematodes by producing complex predatory structures (such as trapping hyphae, adhesive net, constriction loop, or non-constricting adhesive network) [5-7]. Numerous studies have shown that nematode trapping fungi have potential value for the development of biological control agents [8-12]. It has been demonstrated that chitin is an important component of the nematode body wall and the eggshell [13]. Recently, more and more studies have shown that nematode trapping fungi can secrete a variety of extracellular hydrolases, such as serine protease, chitinase and collagenase, to digest and degrade nematode body wall and eggshell, thus penetrating and infecting nematodes or eggs [14,15].

As a typical representative strain of nematode trapping fungi, *Arthrobotrys oligospora* can capture and kill the parasitic nematodes of livestock, and is one of the important fungi for the study of fungi-nematode interaction [16-20]. So far, many scholars have identified and isolated different chitinases from *A. oligospora* [15]. However, the role and mechanism of chitinase in the infection and degradation of nematodes by *A. oligospora* is still unclear.

The aim of this study is to explore the enzymological properties and nematode-degrading activity of recombinant chitinase AO-379 (reAO-379) of *Arthrobotrys oligospora*. Chitinase AO-379 gene of *A. oligospora* was cloned and it's molecular characterization was analyzed. Then reAO-379 was prepared using *Pichia pastoris* expression system, and enzymological properties and nematode-degrading activity of reAO-379 were determined.

MATERIAL and METHODS

Cultivation of the Fungus and Induction of Predatory Hyphae

A. oligospora strain XJ-A1 that was isolated in Xinjiang, and stored in Shihezi University was inoculated in corn meal agar (CMA) medium and incubated at 25°C for 6 days. Appropriate amount of nematode was added to the culture to induce the formation of predatory hyphae (hyphae ring). When the three-dimensional hyphae network was induced by the nematode, the hyphae was collected and stored at -80°C for further study.

Amplification and Cloning of A. oligospora AO-379 Gene

The total RNA was extracted by Trizol reagent (Takara, Japan). The cDNA was reverse transcribed with the reverse transcription kit (Qiagen, Germany). The primer sequences that were used for the amplification of AO-379 cDNA (MG279142) were as follows: forward primer: 5'-GATACGTAATGCATCATCATCATCATCATTTGTTCAAATATAT-3' (containing the *Sna*B1 restriction site); reverse primer: 5'-CGAGCGGCCGCTTAGTTGGACAAGAAGCCC-3' (containing the *Not*1 restriction site). The PCR products were recovered using Agarose Gel DNA Extraction Kit (Takara, Japan) ligated with pMD18-T vector (Takara, Japan) at 16°C overnight, and transformed into *E. coli* DH5α competent cells. The positive transformants were confirmed by PCR and double-enzyme digestion. The screened positive clones were sent to The Beijing Genomics Institute (BGI) for sequencing. The sequencing results were aligned and at least two identical cloning sequences were selected to analyze the molecular characteristics of the encoded protein using the online biology software *(http://www.expasy.org/tools/)*.

Construction and Screening of Recombinant Pichia pastoris

Briefly, the RT-PCR product and the pPIC9K (Invitrogen, USA) were digested with *Sna*B1 and *Not*1 (Takara, Japan), respectively. The vector and the target gene fragments were recovered individually and ligated with T4 ligase (Promega, USA) at 16°C. Then, the ligation product was transformed into *E. coli* DH5α competent cells, and the recombinant vector pPIC9K-AO-379 was screened by PCR and double-enzyme digestion. The pPIC9K-AO-379 was linearized by *Sal* I (Takara, Japan) digestion and transformed into *Pichia pastoris* GS115 (Invitrogen, USA) by electroporation under the conditions of 1.5kV, 20 μF and 200 Ω.

Expression and Purification of Recombinant Protein AO-379 of A. oligospora

The recombinant yeast colony was inoculated into 20 mL BMGY medium (Invitrogen, USA), cultured at 28°C with 250 r/min rotation until $OD_{600\,nm}$ = 2-4. The yeast cells were collected by centrifugation and induced with methanol in 20 mL 0.5% BMMY medium (Invitrogen, USA). The culture medium was collected at 24, 48, 72 and 96 h following induction. The supernatant was collected by centrifugation and analyzed by SDS-PAGE. Take out the purified protein that was expressed in prokaryotic expression system, fully mixed with Freund's complete adjuvant (1:1 dilution), and subcutaneously inoculated in 12 mice (Each mouse was inoculated with 200 μm). Two weeks later, the purified protein was fully mixed with Freund's incomplete adjuvant (1:1 dilution) and the mice were inoculated again. At the third week, take blood through the heart of mice. After

centrifugation (3 000 r/min 10 min), the positive mouse anti-reAO-379 serum was used as the primary antibody and HRP-labeled goat anti-mouse antibody (Abcam, USA) was used as the secondary antibody for Western blot analysis. The recombinant chitinase AO-379 (reAO-379) was purified according to the Ni-NTA Purification System (Invitrogen, USA) and lyophilized.

Analysis of Chitinase Activity of A. oligospora reAO-379

Chitinase hydrolyzes chitin to produce N-acetylglucosamine and produces red compound with p-dimethylaminobenzaldehyde. The chitinase activity of reAO-379 was determined using $OD_{585\ nm}$ absorbance according to the instructions of the chitinase detection kit (Takara, Japan) in culture supernatants that were collected at different induction timepoints. At 37°C, 1 mg chitinase decomposition of chitin in 1 hour could produce 1 mg N-acetylglucosamine, the amount of enzyme was an enzyme activity unit (U). Each experiment was repeated three times.

Determination of Optimum Temperature and pH for A. oligospora reAO-379

According to the methods reported by Wang et al.[13] and Zhao et al.[21] the optimum temperature and optimum pH of reAO-379 chitinase were determined using a protein/nucleic acid analyzer ($OD_{585\ nm}$). Each experiment was repeated three times. Briefly, 40 µL reAO-379 solution was added into 400 µL nematodes and eggs-containing Tris-HCl (0.01 M, pH 7.0). The reaction mixture was incubated at different temperatures between 10°C to 60°C to determine the optimum temperature. The recombinant chitinase AO-379 was added into pH 3.0-8.0 Tris-HCl buffer (0.01 M) to determine the optimum pH of the enzyme.

Analysis of the Nematode-degrading Activity of A. oligospora reAO-379

Twenty-five mg reAO-379 was dissolved in 500 µL Tris-HCl buffer (optimum pH) to prepare 50 mg/mL enzyme solution. Subsequently, about 1.500 *S. equinus*, *C. elegans* and *H. contortus* were added separately into 150 µL reAO-379 enzyme solution and incubated at optimum temperature for 12, 24, and 36 h. The changes in nematode body wall were observed by using inverted microscope (Leica, Germany). Normal saline (NS) and commercialized chitinase (Chitinase from *Streptomyces griseus*, Sigma, USA) solution were used as the negative and positive control, respectively. The number of dead bodies in different solutions that were incubated for different time were observed and calculated. Each experiment was repeated three times.

Analysis of the Nematode Eggshell-degrading Activity of A. oligospora reAO-379

Twenty-five mg reAO-379 was dissolved in 500 µL Tris-HCl buffer (optimum pH) to prepare 50 mg/mL enzyme solution. Then, about 2.000 *F. hepatica* and *D. chinensis* eggs were added individually into 150 µL reAO-379 enzyme solution and incubated at optimum temperature for 24, 48 and 72 h. The changes in eggs were observed by using inverted microscope (Leica, Germany). Normal saline (NS) and commercialized chitinase (Sigma, USA) solution were used as the negative and positive control, respectively. Each experiment was repeated three times.

Statistical Analysis

Statistical analysis was conducted using SAS software (version9.1, SAS Institute, Inc., Cary, NC). A comparison of the killing or degradation rates among different nematodes and eggs was performed using the $\chi 2$-test. The value with $P<0.05$ was considered statistically significant.

RESULTS

The conidia of *A. oligospora* were pointed at the base *(Fig. 1G)*. After 15 days of culture, chlamydospores were

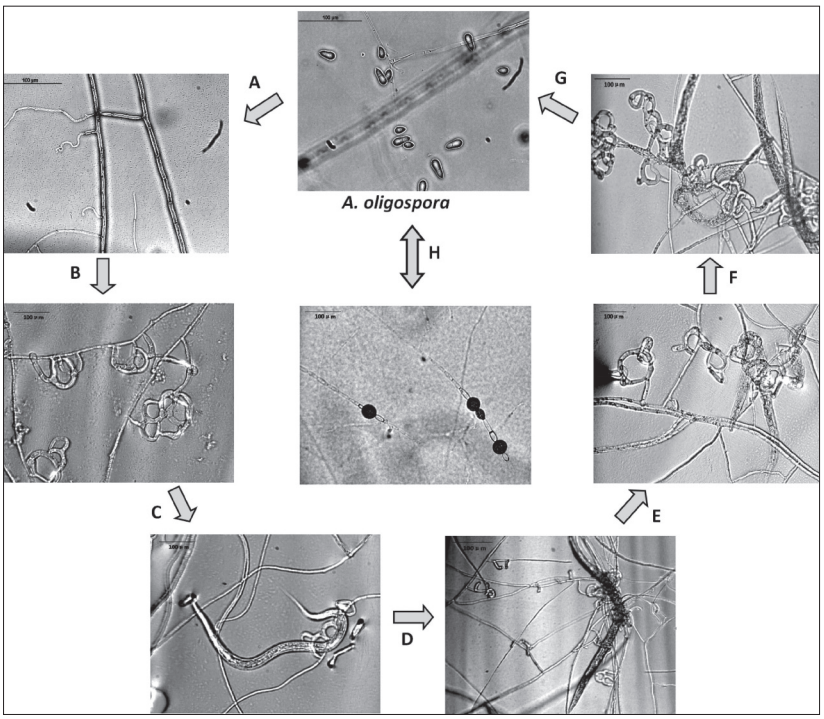


Fig 1. Saprophytic and parasitic stages of the nematode-trapping fungus *A. oligospora.* The life cycle of *A. oligospora* includes three stages: Dormancy stage, saprophytic stage, and parasitic stage. The all stage can be divided into the following eight steps: **A-** The formation of nutrient mycelium; **B-** The formation of the predation ring; **C-** Adhesion of nematodes; **D-** Nematode trapping; **E-** Penetration and immobilization; **F-** Digestion and assimilation; **G-** The saprophytic stage and H. Dormancy stage

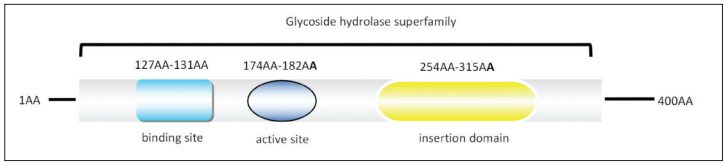
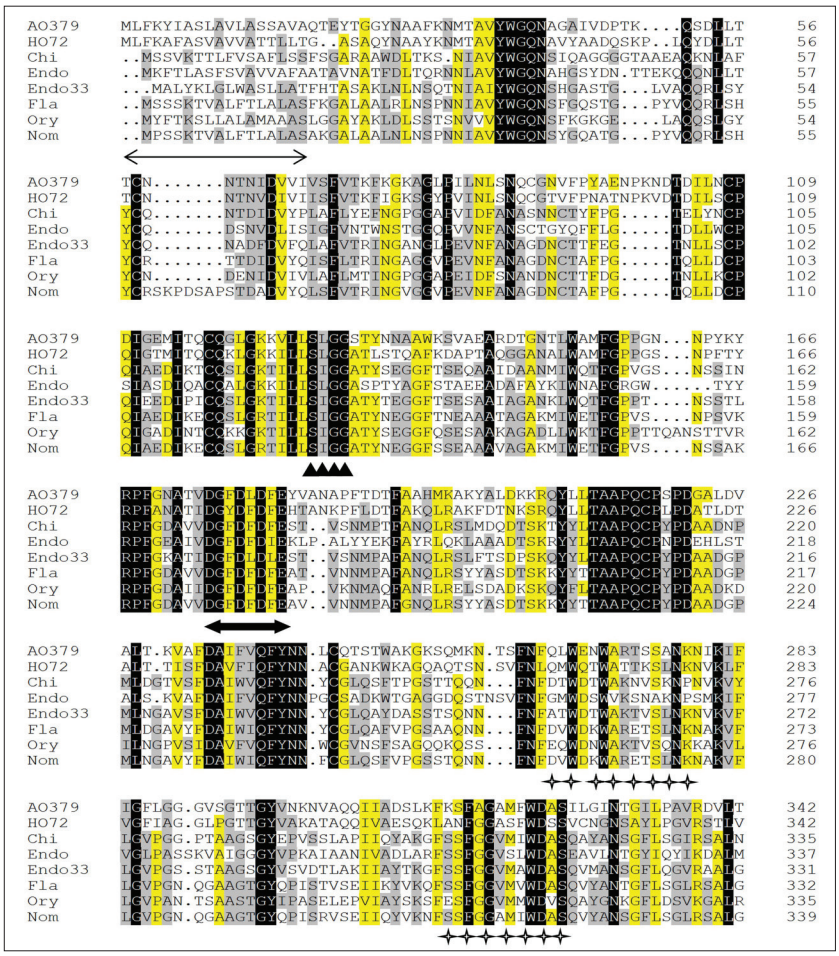


Fig 2. Domain pattern of chitinase AO-379 gene encoding protein of *Arthrobotrys oligospora*

Fig 3. Comparison of amino acid sequence of major catalytic active regions of chitinase among different fungi. The GenBank accession numbers of AO-379, HO72, CHI, Endo, Endo33, Fla, Ory and Nom chitinase from different fungi are MG279142, XP_011110546.1, XP_013323183.1, CCX31601.1, GAQ05774.1, KOC09654.1, XP_001822714.2 and XP_015410999.1, respectively. The black portion is highly homogeneous (100%) of the area; The middle part of the gap between the chitin enzyme variable region; (SXGG, DGXDXDWE) and the binding region of Chitinase (ENGVMDYKA, GLCCGMWWETSG) of the glycosyl hydrolase 18 family of conserved hydrolysates; *Double arrows* denotes a signal peptide; *triple asterisk* represents a substrate binding site; *Left* and *right arrows* represents the active site; *Cross star* represents the chitinase binding domain



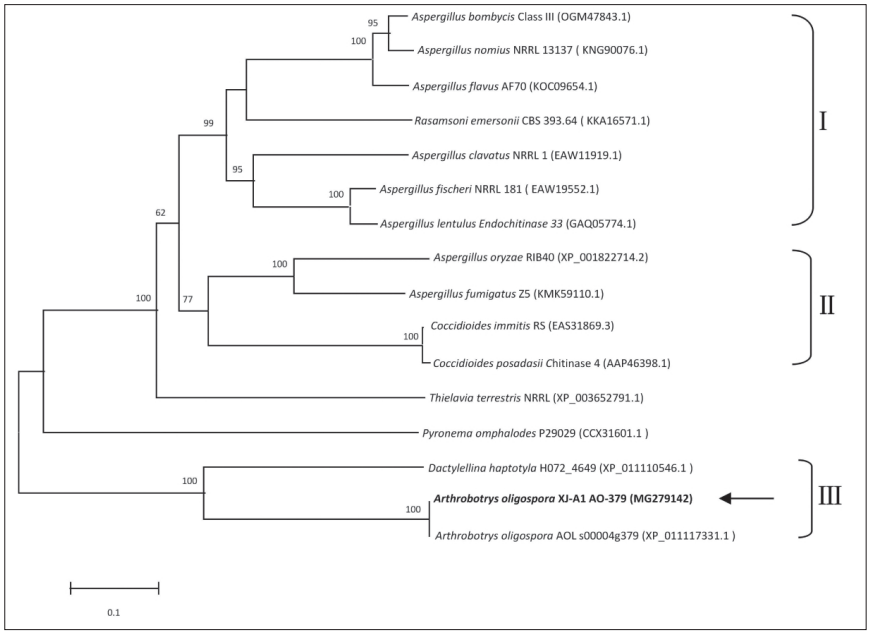


Fig 4. Phylogenetic analysis of chitinases based on amino acid sequences from different fungi. Phylogenetic tree were constructed using MEGA 6.0 software. The data were subjected to neighbor-joining (NJ) method of phylogenetic analysis, and bootstrap probabilities for each node are calculated from 1000 repetitions

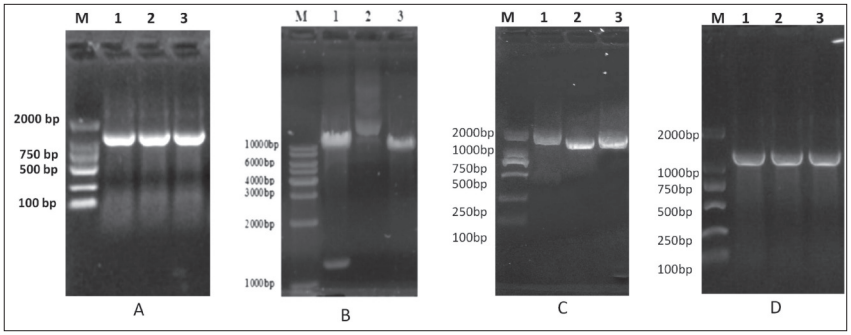


Fig 5. Construction and identification of recombinant plasmid pPIC9K-AO-379 and positive transformants. **A-** PCR amplification of cDNA gene with primers AO-379-F-SnaBI and AO-379-R-NotI using fungal gene/pPIC9K-AO-379 as a template. Lanes 1, 2, and 3 were the PCR products. M was the DNA marker, following lane M is the same; **B-** pPIC9K-AO-379 digested by SnaBI and NotI. Lane 1, empty vector; lane 2, recombinant vector; lane 3, product after enzyme digestion; **C-** PCR amplification of genes (wild-type AOX1 and vector AOX1 with the gene of interest) from Pichia pastoris GS115/pPIC9K-AO-379 using primers 5'AOX1 and 3'AOX1. Lanes 1 and 2 were the PCR products; **D-** PCR amplification of cDNA gene from P. pastoris GS115/pPIC9K-AO-379 using primers AO-379-F-SnaBI and AO379-R-NotI. Lanes 1 and 2 were the PCR products

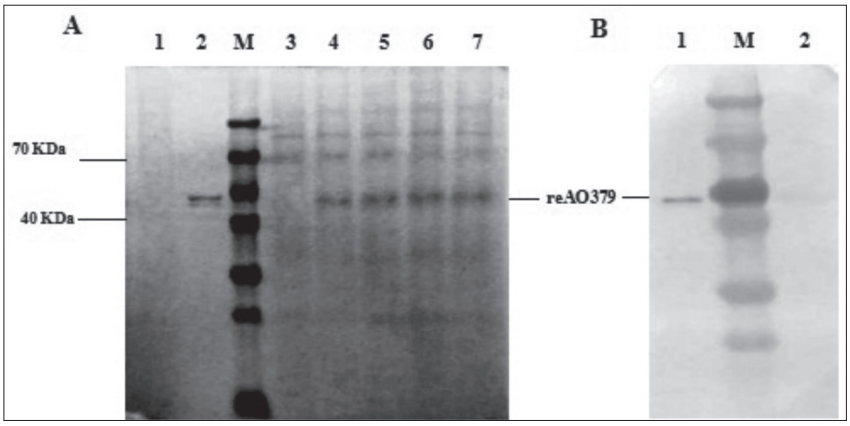


Fig 6. SDS-PAGE and Western blot analysis of expression of reAO-379. **A-** SDS-PAGE analysis of reAO-379. Lane M, protein molecular weight marker (broad). Lane 1: untransformed GS115; lane 2: The purified reAO-379; lane 3: GS115/pPIC9K; lane 4, 5, 6 and 7: GS115/pPIC9K-AO-379 after induction of 24 h, 48 h, 72 h and 96 h, respectively; M: molecular mass standard; **B-** Western blot analysis of reAO-379. Lane 1: purified reAO-379. Lane 2: supernatant from the negative stain transformed with pPIC9K (negative control). Lane M: protein molecular weight marker (broad)

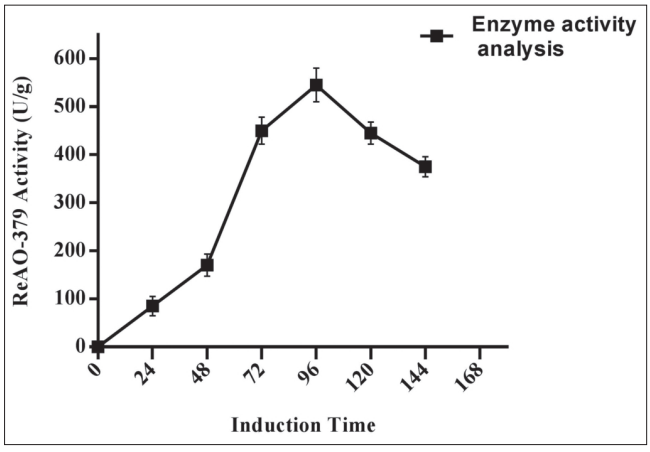


Fig 7. Analysis of chitinase activity of reAO-379 expressed in the cultures of *Pichia pastoris;* The activity of reAO-379 was determined by the reaction of N-acetylglucosamine with p-dimethylaminobenzaldehyde to produce a red compound resulting in a change in absorbance. Results are expressed as A_{585}/min×10^{-3} (unit)

produced in *A. oligospora* *(Fig. 1H)*. After being cultured at 25°C for 24 h, the conidia germinated to form ordinary hyphae *(Fig. 1A,B)*. After 10 h of induction with nematodes, *A. oligospora* began to catch the nematodes *(Fig. 1C)*; The three-dimensional network structure emerged 12 h following induction, the nematodes were trapped by the hyphae ring *(Fig. 1D)*. Fourteen h later, some trapped nematode body shrinked and some hyphae penetrated the nematode body wall *(Fig. 1E)*. Sixteen h later, the contents of the nematodes that were captured were gradually reduced. Finally, the nematodes only contained an empty shell which was ablated eventually *(Fig. 1F)*.

The sequencing result showed that the full length *A. oligospora* AO-379 gene was 1.203bp, encoding 400 amino acids. The signal peptide was located at positions 1-21. The sequence of chitinase AO-379 shared 97.08% homology with the sequence of the *A. oligospora* standard strain (ATCC 24927). Scanprosite software analysis showed that the encoded protein AO-379 belonged to the family of glycoside hydrolase 18, which is characterized by the sequence of VDGFDLDFE at 174-182 amino acids. AO-379 contained two conserved regions: SLGGS was located at positions 127-131 and was the substrate binding site; VDGFDLDFE was located at positions 174-182 and was the hydrolase catalytic activity site *(Fig. 2, Fig. 3)*. Phylogenetic analysis showed that the fungal chitinase formed three major branches. *Aspergillus*-produced seven chitinases formed the first branch; AO-379 belonged to the third branch, which was closest to *Dactylellina haptotyla* chitinase. Endophytic fungi and insect pathogenic fungi produced chitinases (RIB40, Z5, RS and Chitinase 4) formed the second branch *(Fig. 4)*.

The pPIC9K-AO-379 recombinant vector was successfully constructed *(Fig. 5)*. The recombinant *Pichia pastoris* was inoculated in the medium and the recombinant enzyme was induced to express by methanol. After 72 h induction, SDS-PAGE analysis showed that the expressed recombinant protein had a molecular weight of 44 kDa, which was consistent with the expected molecular weight of reAO-379 *(Fig. 6A)*. Western blot showed that the recombinant protein could specifically react with the positive mouse anti-AO-379 serum, confirming that the expressed recombinant protein was reAO-379 *(Fig. 6B)*.

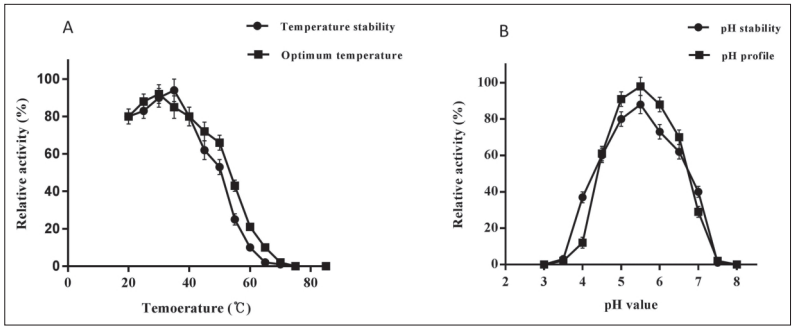
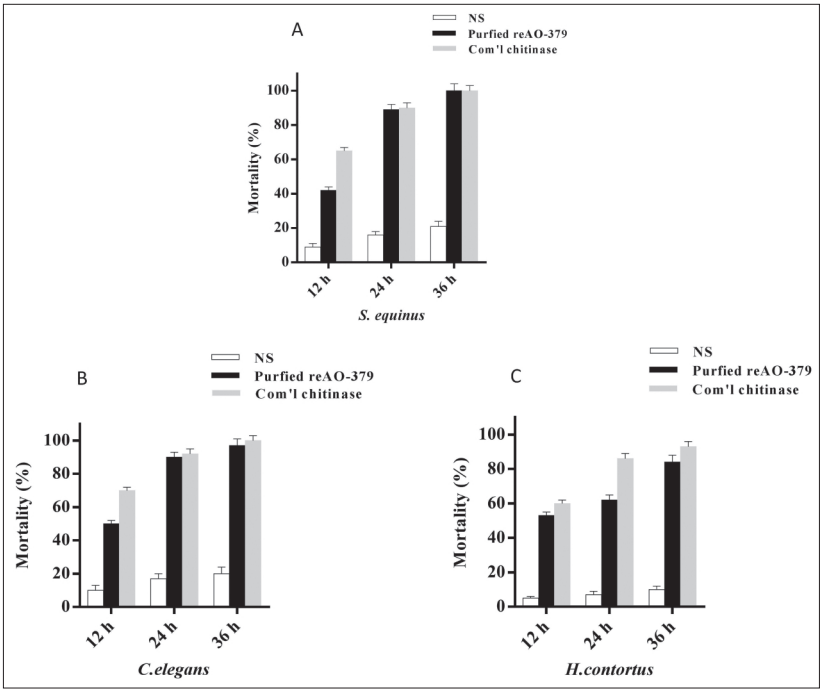


Fig 8. Effects of temperature and pH on activity of purified reAO-379. **A**- Different temperature; **B**- Different pH. Relative activity is expressed as a percentage of the maximum activity under standard assay conditions. The activity of reAO-379 (50 mg/mL) before incubation was defined as 100%. The value is the average of three replications

Fig 9. Analysis of nematicidal activity of the purified reAO-379. **A**- *S. Equinus*; **B**- *C. elegans*; **C**- *H. contortus*. The nematodes in the different time periods were digested by purified reAO-379. The normal saline (NS) commercial chitinase (Com'l chitinase) were used as negative and positive control, respectively. Data represent the average values of three independent assays



The chininase activity of reAO-379 in *Pichia pastoris* culture medium was low during 0-48 h methanol induction, and was increased rapidly after 48 h induction. The chitinase activity reached the maximum (545 U/g) after 96 h induction *(Fig. 7)*, and the chitinase concentration detected in the supernatant of the culture medium was 6.6 mg/mL.

The enzymatic activity of reAO-379 increased gradually with increasing temperature in the range of 10-30°C, and reached the maximum at 30°C. The enzymatic activity of reAO-379 was relatively stable at 20-40°C, while the enzyme was almost inactivated when being incubated at 70°C for 30 min *(Fig. 8A)*. The reAO-379 was active at pH 3.5-7.5, and reached its maximum activity at pH 5.5 *(Fig. 8B)*.

Using reAO-379 to treat *S. Equinus*, *C. elegans* and *H. contortus* separately for 12, 24, and 36 h, we found that the killing rates of reAO-379 in *S. equinus* were 42%, 89% and 100%, respectively *(Fig. 9A)*, in *C. elegans* were 50%, 90% and 97% *(Fig. 9B)*, and in *H. contortus* were 53%, 62% and 84%, respectively *(Fig. 9C)*. The nematode killing rate in AO-379 treated groups was significantly different when compared with the negative control group ($P<0.05$); while it was not significantly different when compared with the commercialized chitinase ($P>0.05$). After 12 h reAO-379 treatment, the nematode cuticle was partially degraded *(Fig. 10A-a4, Fig. 10B-b4, Fig. 10C-c4)*. After 24 h treatment, the body wall of the nematodes was largely degraded *(Fig. 10A-a5, Fig. 10B-b5, 10C-c5)*. After 36 h treatment, the body wall of the nematodes was completely degraded and the internal tissue of the nematodes was destroyed *(Fig. 10A-a6, Fig. 10B-b6, Fig. 10C-c6)*, whereas the body wall of the nematodes without reAO-379 treatment was relatively smooth *(Fig. 10A-a1, a2, a3; Fig. 10B-b1, b2, b3; Fig. 10C-c1, c2, c3)*.

Using reAO-379 to treat *F. hepatica* and *D. chinensis* eggs individually for 24, 48, and 72 h, we found that the killing rates of reAO-379 in *F. hepatica* eggs were 12%, 43% and 65%, respectively *(Fig. 11A)*, and in *D. chinensis* eggs were 15%, 33% and 55%, respectively *(Fig. 11B)*. The nematode eggshells in the negative control group were not degraded at all. After 24 h treatment with reAO-379, the nematode cuticle was slightly degraded *(Fig. 12A-a4, Fig.*

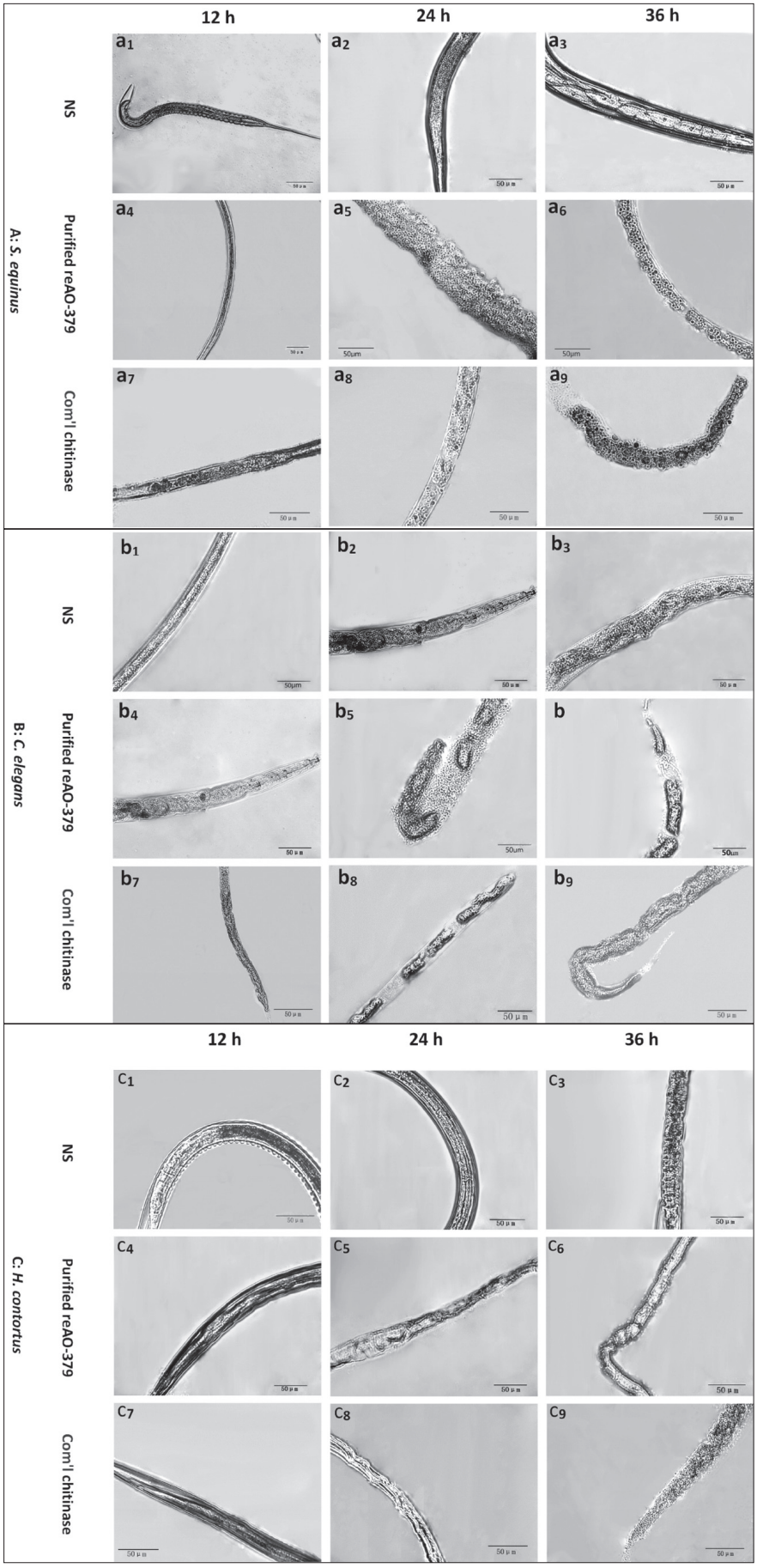


Fig 10. Immobilization and degradation of nematodes treated by purified reAO-379. **A-** *S. equinus* treated by the purified reAO-379 and commercial chitinase were degraded after 12 h, 24 h and 36 h of incubation (a_4, a_5 and a_6), respectively; *S. equinus* treated with the commercial chitinase was degraded after 12 h, 24 h and 36 h of incubation (a_7, a_8 and a_9), respectively; **B-** *C.elegans* treated with the purified reAO-379 and commercial chitinase were degraded after 12 h, 24 h and 36 h of incubation (b_4, b_5 and b_6), respectively; *C. elegans* treated with the commercial chitinase was degraded after 12 h, 24 h and 36 h of incubation (b_7, b_8 and b_9), respectively; **C-** *H.contortus* treated with the purified reAO-379 and commercial chitinase were degraded after 12 h, 24 h and 36 h of incubation (c_4, c_5 and c_6), respectively; *H.contortus* treated with the commercial chitinase was degraded after 12 h, 24 h and 36 h of incubation (c_7, c_8 and c_9), respectively

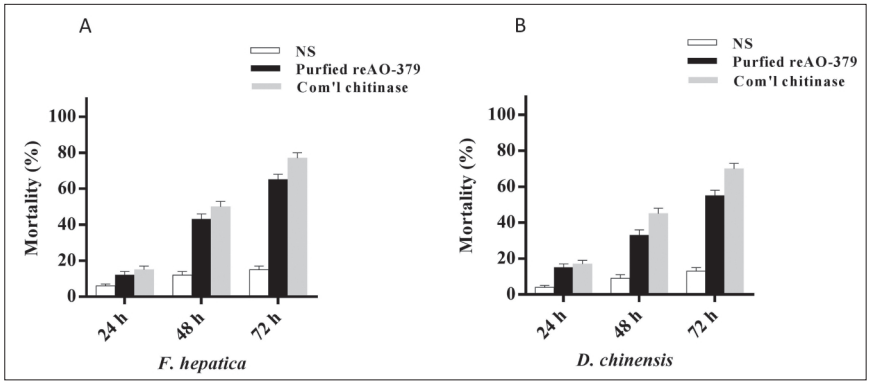


Fig 11. Analysis of degrading activity of the purified reAO-379 in *F. hepatica* and *D. chinensis* eggs. **A-** *F. hepatica*; **B-** *D. Chinensis*. The eggshells were digested in the different time periods. The normal saline (NS) commercial chitinase (Com'l chitinase) were used as negative and positive control, respectively. Data represent the average values of three independent assays

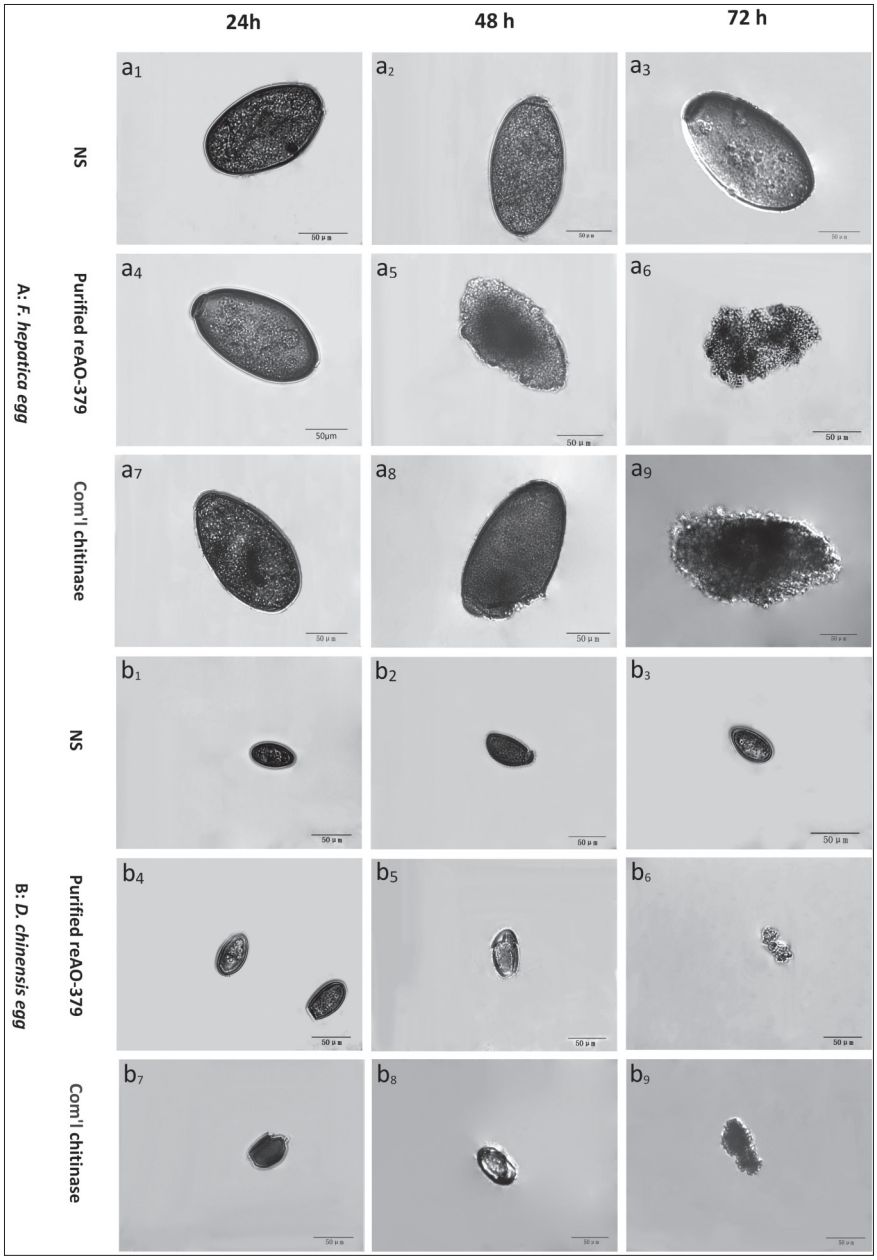


Fig 12. Immobilization and degradation of *F. hepatica* and *D. chinensis* eggs by purified reAO-379. **A-** *F. hepatica* eggs treated with purified reAO-379 was degraded after 24 h, 48 h and 72 h of incubation (a_4, a_5 and a_6), respectively; *F. hepatica* eggs treated with the commercial chitinase (Com'l chitinase) was degraded after 24 h, 48 h and 72 h of incubation (a_7, a_8 and a_9) (positive control), respectively; *F. hepatica* eggs treated with NS was as negative control (a_1, a_2 and a_3); **B-** *D. chinensis* eggs treated with the purified reAO-379 was degraded after 24 h, 48 h and 72 h of incubation (b_4, b_5 and b_6), respectively; *D. chinensis* eggs treated with the commercial chitinase (Com'l chitinase) was degraded after 24 h, 48 h and 72 h of incubation (b_7, b_8 and b_9) (positive control), respectively. *D. chinensis* eggs treated with NS was as negative control (b_1, b_2 and b_3)

12B-b4). After 48 h treatment, the eggshells were almost completely degraded *(Fig. 12A-a5, Fig. 12B-b5)*. After 72 h treatment, the eggshells were degraded and some tissues were destroyed *(Fig. 12A-a6, Fig. 12B-b6)*, but the nematode body without reAO-379 treatment was smoother *(Fig. 12A-a1, a2, a3; Fig. 12B-b1, b2, b3)*.

DISCUSSION

Chitinase is a protease that is widely expressed in bacteria, prokaryotes and eukaryotes and is capable of hydrolyzing beta-1,4-bond in chitin [22]. The chitin degradation activity of chitinase is carried out by a chitinolytic system with synergistic and continuous action, which hydrolyzes chitin into N-acetyl-β-D-glucosamine (GlcNAc) [23,24]. *A. oligospora* is a representative fungus of nematode trapping fungi, which can produce a variety of extracellular proteases to infect nematodes [15]. Here, through analysis of the sequence of *A. oligospora* chitinase AO-379 gene, we found that the gene encoded protein AO-379 contains the characteristic sequence of VDGFDLDFE and belongs to the family of glycoside hydrolase 18 (GH18) [25]. Simltaneously, it also has a substrate binding site and a hydrolase catalytic activity site. Phylogenetic analysis showed that AO-379 is closely related to *Dactylellina haptotyla* chitinase, but is far from the chitinases of endophytic fungi and insect pathogenic fungi.

Tunlid et al.[26] purified serine protease PII from *A. oligospora* and confirmed that it can degrade the cuticle of the nematode body wall. Subsequently, chitinase was found in various fungi [27-29]. At present, 15, 18, 21 and 30 chitinases have been identified in the genomes of pathogenic fungi (such as *M. oryzae*, *T. reesei*, *M. acridus* and *M. anisopliae*); ten chitinases have been identified individually in the genome of non-pathogenic *A. nisulans* and *N. crassa* [27-30]. Yang et al.[15] for the first time, identified 16 chitinase-encoding ORFs from the genome of *A. oligospora*. Transcriptome analysis results show that at carbon/nitrogen deficiencies or in the presence of chitin, the expression level of chitinase AO-379 was increased by 1.64 times, suggesting that AO-379 may play an important role in nematode infection in *A. oligospora* [15].

As an important biocontrol resource for livestock parasitic nematodes, fully understanding *A. oligospora* infection mode and its virulence factor is the key to reveal its infection mechanism and to develop efficient and stable biocontrol agents against nematodes [31]. Current studies have shown that nematode trapping fungi can secrete extracellular proteases that degrade the cuticle of nematode body wall or the eggshells [13], which may play a very important role in nematode infection [32]. In order to elucidate the biological function of *A. oligospora* chitinase AO-379, we expressed the chitinase AO-379 gene in *Pichia pastoris*. The recombinant AO-379 (reAO-379) was purified by nickel ion affinity chromatography. SDS-PAGE analysis showed that the reAO-379 with molecular weight of about 44 kDa was successfully obtained. Western blot showed that the recombinant protein could specifically react with the positive mouse anti-AO-379 serum, confirming that the expressed recombinant protein was reAO-379 Enzymatic activity analysis results showed that the activity of purified reAO-379 was high and stable at 30°C and pH 5.0-6.0, with the maximum activity at pH 5.5. When treated with reAO-379, the body wall of *S. equinus*, *C. elegans* and *H. contortus* was almost completely degraded and some of the internal tissue was also destroyed. The eggshells of the nematodes *F. hepatica* and *D. chinensis* were completely degraded after being treated with reAO-379 for 72 h.

In conclusion, we analyzed the molecular characteristics of chitinase AO-379 of *A. oligospora*, heterologously expressed AO-379 in *Pichia pastoris* and demonstrated that reAO-379 had strong biological activity to degrade the nematode body wall and eggshell, which provides the theoretical basis for the further development of nematode biological control agents using reAO-379.

Supplementary Date

Supplementary data are available in supplementary files.

Funding

This work was supported by National Natural Science Foundation of China (No. 31460654, 31260601), National Key Research and Development Program of China (No. 2017YFD0501200), Grant from Youth Science and Technology Innovation Leader of Xinjiang Production and Construction Corps (No. 2016BC001) and Graduate Student Research Innovation Project of Xinjiang Autonomous Region (No. XJGRI2015038).

Ethical Statement

Ethical approval for this study was given by the Research and Ethical Committee of Shihezi University.

Declaration of Conflicting Interests

This manuscript has not been simultaneously submitted for publication in another journal and been approved by all co-authors. The authors declare that they do not have any conflict of interest.

Acknowledgments

We thank Professor Du Aifang in Zhejiang University who provided the parasites of *C. elegans*, *H. contortus* and *S. equinus* for this study.

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