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Full Length Article

Molecular Characteristics and Adhesion Activity of a Novel Protein ADP1 of *Arthrobotrys oligospora* to Nematodes

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Abstract

Adhesion is a crucial step for nematode-trapping fungi (NTF) predating nematodes. To investigate the function of a novel protein *ADP1* in nematode-trapping process, *ADP1* gene of a representative NTF-*Arthrobotrys oligospora* was cloned and the molecular characteristics of this protein were analyzed. Then, the *GFP* chimeric *ADP1* (*ADP1-GFP*) was generated in a *GFP* expression vector and expressed in *Escherichia coli* BL21 (DE3) and the recombinant *ADP1-GFP* (*reADP1-GFP*) was purified. Incubation of *reADP1-GFP* with J3 larvae of *Caenorhabditis elegans* and *Haemonchus contortus* showed that *reADP1-GFP* could adhere nematodes with the strongest adhesion ability at 25°C, while the *reADP1-GFP* treated by trypsin completely lost the adhesion ability. Furthermore, the numbers of captured nematodes of *A. oligospora* treated by anti-*reADP1-GFP* serum in the experimental group was significantly lower than that in the control group, which suggests that the nematode-trapping activity of *A. oligospora* is inhibited by anti-*ADP1* serum. To the best of our knowledge, this is the first report confirming that ADP1 from *A. oligospora* can modulate the adherence to *C. elegans* and *H. contortus*. The exploration of interaction between *ADP1* protein of *A. oligospora* and nematodes provides new insights into the process of invasion and molecular mechanisms of *A. oligospora* preying nematode. © 2021 Friends Science Publishers

Keywords: *Arthrobotrys oligospora*; Adhesion protein; Fusion protein; ADP1-nematode interactions

Introduction

Gastrointestinal nematode disease of livestocks is parasitic disease seriously threatening the development of livestock industry and annually causing huge economic losses (Sréter *et al*. 1994; Tembely *et al*. 1997; Kaewthamasorn and Wongsamee 2006; Terrill *et al*. 2012). Currently, the disease is mainly prevented and controlled by chemical drugs. However, long-term use of these chemical drugs at high dosage has drawbacks (drug resistance, drug residues and environmental pollution) and becomes an increasingly prominent issue (Hay *et al*. 1997; Alvarez *et al*. 2008). Therefore, it is necessary to seek animal- and environment-friendly prevention and control methods. Using nematode predators-nematode-trapping fungi (NTF) to achieve the goal is considered as a prospective biological method (Grønvold *et al*. 1993; Gives and Vazquez-Prats 1994; Bird and Herd 1995; Chandrawathani *et al*. 1998; Fernández *et al*. 1999; Flores-Crespo *et al*. 2003).

NTF, are class fungi of more than 700 species that are able to prey, parasite or colonize nematodes. As the natural nematode predators, NTF can produce predatory organs to capture nematodes, most of their preying processes include identification, attraction, adhesion and degradation (Nordbring-Hertz *et al*. 2006), among which, adhesion is the most important step for preying nematodes. However, so far, the underlying molecular mechanisms of NTF preying nematodes are still incompletely understood (Liang *et al*. 2013; Andersson *et al*. 2014; Liu *et al*. 2014).

In recent years, the genomes of a number of NTF have been successfully sequenced and their genes related to predation have been studied in depth (Liu *et al*. 2018; Liang *et al*. 2013). As a representative of predatory fungi of nematode species, the genome of *Arthrobotrys oligospora* was first sequenced in 2011. Based on the results, Yang *et al*. (2011) predicated 17 adhesion-related protein-coding genes and found by qPCR that one of the predicted proteins, named *ADP1*, was upregulated by 21.7-fold during their

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predatory organ formation, suggesting that *ADP1* may play an important role in the process of *A. oligospora* trapping nematode (Yang *et al*. 2011). However, the molecular characteristics and function of *ADP1* of *A. oligospora* is still uncovered. The aim of this study is to analyze the molecular characteristic of a novel *ADP1* protein of *A. oligospora*, and to explore the roles of *ADP1* protein in the process of nematode-trapping, thus understanding the biological function of *ADP1* of *A. oligospora* in invading nematodes.

Materials and Methods

Amplification of *ADP1* gene of *A. oligospora*

Based on the full-length *A. oligospora ADP1* gene sequence with accession number AOL_s00210g23 in GenBank published by Yang *et al*. (2011), a pair of ADP1 specific primer P1 and P2 was designed. After cultured in liquid LMZ medium (Tiangen, China) at 26°C with shaking at 150 rpm for 3 d, *A. oligospora* XJ-A1 strain was collected and its total RNA was extracted using Trizol (Invitrogen, USA) and reversely transcripted into cDNA using PrimeScript™ reagent kit (Takara, Japan). The cDNA was then used as the template to amplify *ADP1* gene at PCR reaction conditions of 95°C for 5 min followed by 30 cycles of 40 s at 94°C, 40s at 64°C and 1 min at 72 and final 10 min at 72°C.

Cloning of *ADP1* gene from *A. oligospora*

The obtained *ADP1* gene was recovered using Agarose Gel DNA Fragment Recovery Kit (Takara, Japan) and cloned into pMD18-T vector (Takara, Japan). The correct clones were identified by PCR and digestion with *Eco*RI and *Bam*HI and further verified by sequencing (BGI, Shenzhen). Four positive clones were sequenced and compared with *A. oligospora ADP1* gene sequence in GenBank.

Analysis of molecular characteristics of *ADP1* protein of *A. oligospora*

The amino acid sequence of *ADP1* was deduced, and its signal peptide was analyzed by software SignalP 4.1 (https://www.cbs.dtu.dk/services/SignalP/). The transmembrane and domains of this protein were predicted by TMHMM 2.0 and Scanprosite software (https://www.expasr.org/), respectively. Moreover, the secondary and tertiary structures were also predicted by Software Sopma and Swiss-model (https://www.expasr.org/), respectively.

Expression and purification of recombinant protein *ADP1-GFP*

The obtained plasmid pT-ADP1 and the expression vector pET28a-GFP were digested with restriction enzymes *Eco*RI and *Hin*d III, respectively, and the digested vector and targeted *ADP1* fragment were ligated at 16°C to generate pET28a-GFP-ADP1 recombinant expression vector. The pET28a-GFP-ADP1 and pET28a-GFP plasmids were identified by PCR using specific primers P1-P2 and P1-P4, respectively, and then transformed into *E. coli* BL21 (DE3) for expression. After 6 h of IPTG (Takara, Japan) induction, cell lysates were subjected to 12% SDS-PAGE analysis. Then, Western blot analysis was performed by using the mouse anti-*reADP1* antibody as the primary antibody and HRP-labeled goat anti-mouse antibody (Abcam, USA) as the secondary antibody. The expressed recombinant proteins *reADP1-GFP* and *reGFP* were purified using Ni-NTA Spin Kit (Qiagen, Germany) according to the instructions provided by the manufacturer, concentrated with millipore ultrafiltration system (Amicon, USA) and adjusted to 1 mg/mL 0.01 M PBS, pH 7.2 solution for future use.

Analysis of interactions between *reADP1-GFP* and nematode

Briefly, the infective larva of *Caenorhabditis elegans* and *Haemonchus contortus* were prepared as suspensions of 2000 nematodes per mL. Then, 1 mL of the larval suspension of *C. elegans and H. contortus* were incubated with 1 mL of *reADP1-GFP*, *GFP*, bovine serum albumin (BSA) and trypsin-treated *reADP1-GFP* at 25°C for 1 h, respectively. Then, 200 μL of each mixture was taken out and centrifuged at 6000 rpm for 1 min and the collected nematodes were washed with 0.01 M PBS, pH 7.2 for 6 times to be observed under a fluorescent microscope.

Effects of anti-*ADP1* antibody on the nematode-trapping activity of *A. oligospora*

The hyphae were transferred to corn meal agar (CMA) solid medium (17 g corn meal, 10 g agar and 2 g K_2 HPO_4 in 1 L of water, adjusted to pH 7 using 1 M NaOH) containing 0.2% rabbit anti-*A. oligospor*a serum, and cultured at 26°C in light-free condition. After 3 days of culture, larval suspension (100 strips) of *H. contortus* was added to the plate. The traps and captured nematodes were counted under a light microscope after 12, 24, 36 and 48 h, respectively. The numbers of traps and captured nematodes were calculated according to the references (Zhao *et al*. 2014; Zhang *et al*. 2017).

Statistical analyses

Statistical analyses were conducted using S.A.S. software Version 9.1 (S.A.S. Institute, Inc., Cary, NC, USA). A comparison of the number of captured nematode between different groups was performed using the Chi-square test. The values of $P < 0.05$ were considered as statistically significant, while $P < 0.01$ as an extremely significant difference.

Results

cDNA of *ADP1* gene amplified from *A. oligospora* by RT-PCR was about 500 bp (Fig. 1). The sequencing results showed that the complete length of *ADP1* gene was 468 bp, which encoded 155 amino acids (Fig. 2). The sequences of *ADP1* gene from *A. oligospora* XJ-A1 strain had been submitted to GenBank under accession numbers MT995855. The *ADP1* gene shared 96.37% identities in nucleotide and 94.19% identities in amino acid, respectively, when it was compared with the corresponding gene (AOL_s00210g23) of *A. oligospora* deposited in GenBank. The *ADP1* protein did contain signal peptide but owned a transmembrane region at amino acids 93-115 of this protein. Analysis of SWISS-MODEL software revealed that *ADP1* formed a cylindrical tertiary structure (Fig. 3).

The recombinant *GFP-ADP1* (*reADP1-GFP*) and recombinant GFP (*reGFP*) proteins expressed in pET28a-GFP-ADP and pET28a-GFP transformed *E. coli* DE3 strain after 6 h of induction with IPTG, showed the expected sizes of 50 kDa and 30 kDa, respectively (Fig. 4 and 5) on SDS-PAGE. Western blot analysis showed that the expressed 50 kDa recombinant protein could interact with rabbit anti-*ADP1* serum, confirming the successful expression of *reADP1-GFP* (Fig. 2 and 4). SDS-PAGE analysis showed that the *reADP1-GFP* and *reGFP* purified with Ni-NTA affinity column had very high purity (Table 1; Fig. 5).

The collected *C. elegans* and *H. contortus* after incubation with purified reADP1-GFP for 1 h at 25°C showed green fluorescence on their surface, whereas those incubated with *reGFP* and BSA showed no green fluorescence on their surface under a fluorescence microscope (Fig. 6), confirming that only *reADP1-GFP* has adhesion activity on nematode surface. In contrast, *reADP1-GFP* treated by trypsin lost its adhesion activity to the surface of nematode when compared to *reADP1-GFP* group, while PBS-treated *reADP1-GFP* did not reduce its adhesion activity to nematode (Fig. 6).

A. oligospora treated by anti-*reADP1-GFP* serum in the experimental group could produce three dimensional nets and capture nematodes as control group (Fig. 7A-D). Compared with the control group, there was no significant differences in the numbers of trap devices between experimental and control group ($P > 0.05$) (Fig. 7E). However, when treated by anti-*reADP1-GFP* serum for 48 h, the numbers of captured nematodes of *A. oligospora* treated by anti-*reADP1-GFP* serum in the experimental group was significantly lower than that in the control group ($P < 0.05$) (Fig. 7F), which suggested that the nematode-trapping activity of *A. oligospora* could be inhibited by anti-*ADP1* serum.

Discussion

As a model of NTF, *A. oligospora* enters the parasitic stage by forming complex three-dimensional networks to trap nematodes (Zhao *et al*. 2014). The trapping initiates a series of processes including adhesion, penetration, and immobilization of nematodes (Tunlid *et al*. 1994; Ahman *et al*. 1996; Minglian *et al*. 2004; Nordbring-Hertz *et al*. 2006; Yang *et al*. 2013; Liang *et al*. 2015; Liu *et al*. 2020). Adhesion is a premise for NTF preying nematodes. The research has shown that the adhesion process of NTF on *C. elegans* is a complex process requiring participation of carbohydrates, proteins, as well as their complexes and other substances (Tunlid and Jansson 1992). Nordbring-Hertz *et al*. (2006) found that there were adhesion

Table 1: List of primer sequences used in this study

Primer name	Nucleotide sequence (5'→ 3')	Target gene	Product size (bp)
P1	CCGGAATTCATGTGTAAACCCTTCGAAATCG	ADP1	468
P1	CCCAAGCTTTCATTTGACTTCATTAAGCTGCC		
P3	ATGAGTAAAG GAGAAGAACTTTTCAC	GFP	714
P4	TTTGTGTCCAAGAATGTTTCCATC		

Note: The underlined sequences in P1 and P2 are the restriction sites of endonucleases *Eco*RI and *Hin*d III, respectively

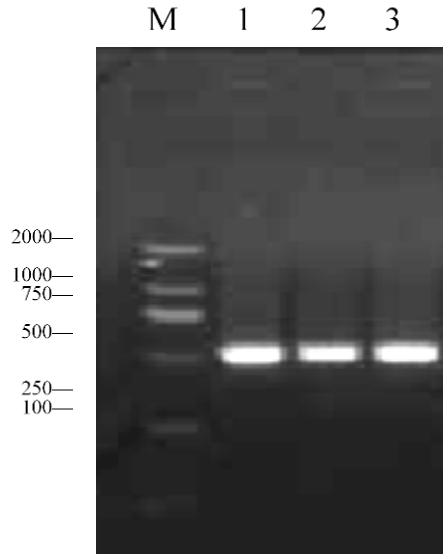

Fig. 1: Amplification of *ADP1* gene of *Arthrobotrys oligospora* by RT-PCR
M: DNA marker (DL-2000), Lanes 1-3: RT-PCR products of *ADP1* gene

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1     ATGTGTAAACCCTTCGAAATCGATCAGACAGTTGGTGCAGTCCGAGAACTATGCGCCGGG
1      M  C  K  P  F  E  I  D  Q  T  V  G  A  V  R  E  L  C  A  G
61    GTCGGAGTTAGCCTGACTATCACGACAACGTGGAGTACAACCCAATCGTCCACAACAGCC
21     V  G  V  S  L  T  I  T  T  T  W  S  T  T  Q  S  S  T  T  A
121   CCGCTCTCGCATCGCCCGTCGGAGACGGCAAGCTCTTCAGAATCATTGATTACACCGGCT
41     P  L  S  H  R  P  S  E  T  A  S  S  S  E  S  L  I  T  P  A
181   ACTACGAACCCCAACTCCGAAGCTACGCCAAGCGATACCTCTACACCTGAAACCCGAGGA
61     T  T  N  P  N  S  E  A  T  P  S  D  T  S  T  P  E  T  R  G
241   AAAGCGGCAGGCGGCTCCAAGTTAAGTGCTGGAGCGATTGCTGGAGTTACTATTGGAGTT
81     K  A  A  G  G  S  K  L  S  A  G  A  I  A  G  V  T  I  G  V
301   ACCGTCCCGGTGGTAGCATTAGTAGGATTTATCTTCATATTATTCCGAAGAAAAGGTAGA
101    T  V  P  V  V  A  L  V  G  F  I  F  I  L  F  R  R  K  G  R
361   AGACTCCACGTCCCTGTTTTATCGACTAGTCAGAGTCACGAGACCAATAATTGGGGTGGG
121    R  L  H  V  P  V  L  S  T  S  Q  S  H  E  T  N  N  W  G  G
421   ATTGGGCCAGATAATGACATTCCCGGGCAGCTTAATGAAGTCAAATGA
141    I  G  P  D  N  D  I  P  G  Q  L  N  E  V  K  *
```

Fig. 2: Nucleotide sequence and amino acids of *ADP1* protein
Note: The different amino acids were underlined; the amino acids constituting transmembrane region were shadowed

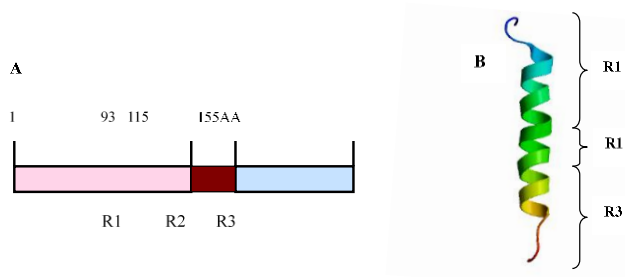

Fig. 3: Schematic diagram of molecular characteristics of *ADP1* protein of *Arthrobotrys oligospora*
A: Outside, transmembrane and inside regions of *ADP1* protein
B: Tertiary structure of *ADP*1 protein
Note: R1: Outside region of membrane; R2: Transmembrane region; R3: Inside region of membrane

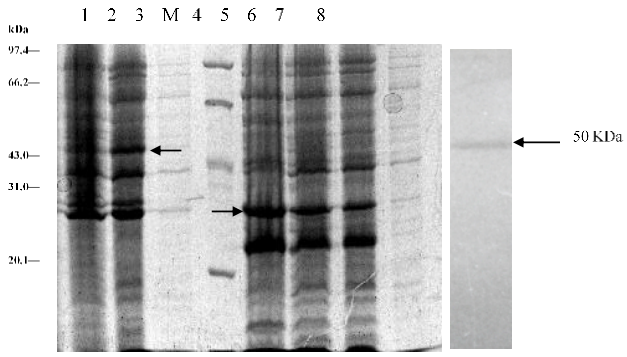

Fig. 4: SDS-PAGE and western blot analysis of the *reADP1-GFP* and *reGFP*
M: Standard protein marker (97.4, 66.2, 43.0,31.0, 20.1 kDa); Lanes 1 and 2: Cell lysates of pET28a-GFP-ADP1 transformed *E. coli* after induced with IPTG for 4 and 6 hours, respectively
Lanes 3 and 7: Cell lysates of pET28a transformed *E. coli* after induced with IPTG for 4 and 6 hours, respectively; Lanes 4, 5 and 6: Cell lysates of pET28a-GFP transformed *E. coli* after induced with IPTG

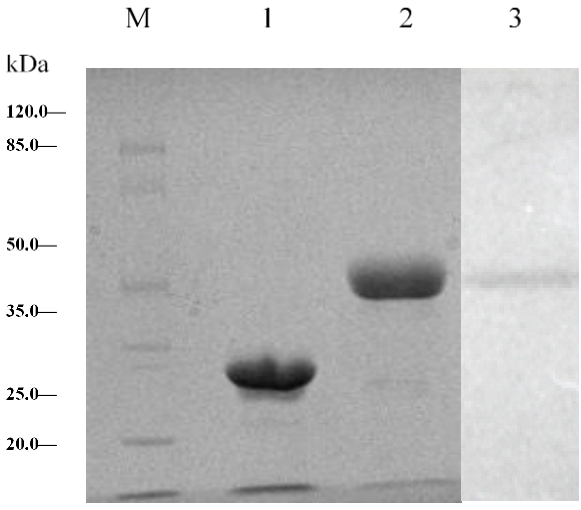

Fig. 5: SDS-PAGE and Western blot analysis of the *reADP1-GFP* and *reGFP*
M: Standard protein marker (120.0, 85.0, 50.0, 35.0, 25.0, 20.0 kDa)
1: Purified *reGFP* protein
2: Purified *reADP1-GFP* protein
3: Western blot analysis of *reADP1-GFP* protein

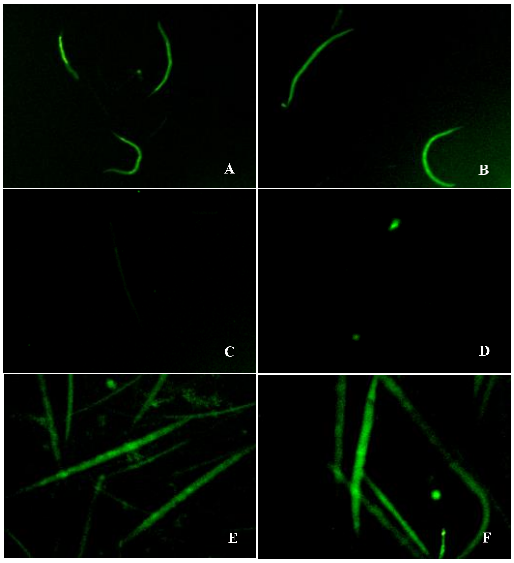

Fig. 6: Analysis of **i**nteraction of *reADP1-GFP* with nematodes
A: *reADP1-GFP* interacts with *C. elegans*
B: *reADP1-GFP* interacts with *H. contortus*
C: BSA interacts with *H. contortus*
D: Trypsin treated *reADP1-GFP* interacts with *H. contortus*
E: PBS treated *reADP1-GFP* interacts with *H. contortus*
F: *reADP1-GFP* interacts with *H. contortus*

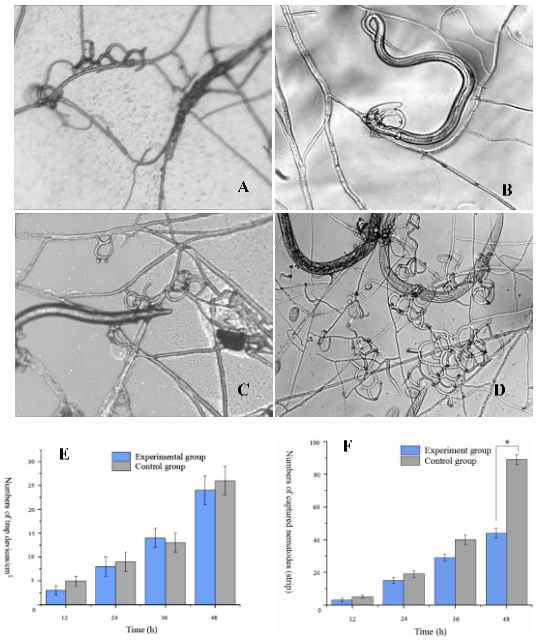

Fig. 7: Effects of anti-*reADP1-GFP* serum on the nematode-trapping activity of *A. oligospora*
A-D: The trap devices in 12 h, 24 h, 36 h and 48 h post-induction of larval suspension of *H. contortus*
E: The numbers of trap devices; **F**: The numbers of captured nematodes

substances between NTF and nematodes and confirmed that adhesion substances contain lectin. Meerupati *et al.* (2013) revealed that certain proteins also play important roles in the adhesion process. Yang *et al.* (2011) conducted whole genome analysis of *A. oligospora* and predicted that 17 genes were related to adhesion, among which, five genes

were upregulated during the formation of their predatory organs, suggesting that some proteins may play important roles in the process of *A. oligospora* adhering to nematode (Yang *et al*. 2011). However, to date, the active adhesion substances produced by NTF and their underlying molecular mechanisms for adhesion are still incompletely understood (Meerupati *et al*. 2013; Liang *et al*. 2013).

Based on the studies on genomics and proteomics of *A. oligospora*, many new functional proteins have been identified and characterized (Li *et al*. 2016, 2017; Liang *et al*. 2017; Xie *et al*. 2019; Yang *et al*. 2018 Zhang *et al*. 2019). To better understand the biological functions of ADP1, interactions between *A. oligospora ADP1* and nematode were conducted. The results revealed that the *reADP1-GFP* protein could adhere to the surface of nematode and was unable to be washed away by elution buffer, suggesting that *ADP*1 has adhesion function to nematodes. Furthermore, we confirmed that *ADP1* displayed stronger adhesion at 25°C, which is in consistence with the natural environment of fungi, suggesting production of *ADP1* may be an environmental adaptability of fungi in the evolutionary process to form a favorable environment for its predation under natural conditions. In addition, trypsin digestion could block the adhesion ability of *reADP1-GFP* protein to nematode, while PBS did not affect its adhesion activity to the surface of nematode, which suggests that this novel protein ADP1 is involved in an adhesion role. The nematode-trapping activity of *A. oligospora* inhibited by anti-*ADP1-GFP* serum further confirmed that the *ADP1* protein was closely related to nematode-trapping process.

Conclusion

A. oligospora ADP1 exerts an important role in the process of fungal adherence to nematodes, which provides new insights into our understanding of the molecular mechanisms of NTF preying nematodes.

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Author Contributions

Li Jie and Meng Qingling planned and designed the whole study. Chen Shuangqing, Li zhiyuan, Wang Lixia, Shang Yunxia, Gong Shasha, Xiao Chencheng, Zhang Kai performed and completed the experiments. Li Jie, Qiao Jun and Meng Qingling wrote the manuscript. Zhang Xingxing and Cai Xuepeng reviewed and revised the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

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.

Data Availability

Data presented in this study are available on fair request to the corresponding author.

Ethics Approval

The experiments were carried out in accordance with the guidelines issued by the Ethical Committee of Shihezi University.

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