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ORIGINAL PAPER

# Cloning and characterization of an extracellular serine protease from the nematode-trapping fungus *Arthrobotrys conoides*

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**Abstract** An extracellular serine protease (Ac1) with a molecular mass of 35 kDa was purified from the nematode-trapping fungus *Arthrobotrys conoides*. The optimum activity of Ac1 is at pH 7.0 and 53.2°C (over 20 min). Ac1 can degrade a broad range of substrates including casein, gelatin, bovine serum albumin, collagen, and nematode cuticles. Moreover, the enzyme can immobilize the free-living nematode *Panagrellus redivivus* and the pine wood nematode *Bursaphelenchus xylophilus*, indicating Ac1 may be involved in infection against nematodes. The encoding gene of Ac1 contains one intron of 60-bp and two exons encoding a polypeptide of 411 amino acid residues. The deduced polypeptide sequence of Ac1 showed a high degree of similarity to two previously reported serine proteases PII and Mlx from other nematode-trapping fungi (81% aa sequence identity). However, three proteases Ac1, Aoz1 and Mlx showed optimum temperatures at 53.2, 45 and 65°C, respectively. Compared to PII, Ac1 appears to have a significantly higher activity against gelatin, bovine serum albumin, and non-denatured collagen. Moreover, our bioassay experiments showed that Ac1 is more effective at immobilizing *P. redivivus* than *B. xylophilus*.

**Keywords** *Arthrobotrys conoides* · Protease purification · Nematicidal activity · Gene cloning · Sequence analysis

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## Introduction

The genus *Arthrobotrys* Corda is one of the most interesting genera of nematode-trapping fungi and produces specialized adhesive networks to capture nematodes (e.g. Dackman and Nordbring-Hertz 1992; Tunlid et al. 1994; Zhao et al. 2004). Adhesion of nematodes to the traps and the penetration process have been studied and described in many species (e.g. Dackman and Nordbring-Hertz 1992; Tunlid et al. 1992). The infection processes against nematodes by nematophagous fungi have been assumed to be a combination of mechanical forces and hydrolytic enzymes (Lopez-Llorca 1990; Segers et al. 1994; Tunlid et al. 1994; Bonants et al. 1995). Among the hydrolytic enzymes, serine proteases have recently been shown to be very important in the penetration and digestion of nematodes by nematode-trapping fungi (Åhman et al. 2002; Lopez-Llorca et al. 2002; Yang et al. 2005a; Wang et al. 2006).

Pathogencity-related proteases PII and Mlx have been identified from nematode-trapping fungi *Arthrobotrys oligospora* (Tunlid et al. 1994) and *Monacrosporium microscaphoides* (Wang et al. 2006), respectively. Previous works on PII and Mlx have mainly focused on identifying and confirming their roles in the infection process. Other information, e.g. substrate and host preference of serine proteases, remained largely unknown. Moreover, the identification of pathogenicity-related proteases from different nematode-trapping fungi will help us find certain key information on host preference, substrate selection, and pathogenictiy-related domains at the protein level. Such information will be important for improving the efficacy of nematophagous fungi in biological control applications (Åhman et al. 2002; Morton et al. 2003).

*Arthrobotrys conoides* Drechsler is an autochthonic fungus, which can immobilize the free-living nematode

*Panagrellus redivivus* and the pine wood nematode *Bursaphelenchus xylophilus* by adhesive network in our preliminary test. In order to determine infection-related factors, we identified a serine protease (Ac1) from *A. conoides*, and its biochemical properties were characterised. The biochemical properties and deduced peptide sequence of Ac1 were compared with two other pathogenicity-related proteases PII and Mlx, identified previously from two closely related nematode-trapping fungi *A. oligospora* and *M. microscaphoides* (Tunlid et al. 1994; Wang et al. 2006), respectively. In addition, we examined the potential host preference of Ac1 between the free-living nematode *P. redivivus* and the pine wood nematode *B. xylophilus*.

## Materials and methods

### Organisms and growth conditions

The isolate of nematode-trapping fungus *A. conoides* (YMFC1.00039) used in this study was originally isolated from a field soil sample in Yunnan Province in China and had been deposited in Yunnan Microbiological Fermentation Culture Collection Center (YMFC). It was incubated on potato dextrose agar (PDA) at 26°C for 6–8 days, and then stored at 4°C. An inducing medium (PL-4) was prepared according to that described in a previous report (Yang et al. 2005a) and was used for producing protease.

*Panagrellus redivivus* and *B. xylophilus* (permanently stored in YMFC) were cultured and maintained according to the method described by Dong et al. (2004). Nematodes were separated and washed thoroughly with 50 mM sodium phosphate buffer (pH 7.0) before being used in the assays.

### Protease activity analysis

A semi-quantitative casein-plate method described by Zhao et al. (2004) was used to determine the protease activities of the supernatant liquid and various purification fractions. Quantitative analysis of protease activity was determined by a caseinolytic method (Walter 1984) with modification (Wang et al. 2006).

### Purification of protease Ac1

After 6 days of growth in the PL-4 medium, the fungal cultures were filtered and subsequently concentrated 20-fold by ultrafiltration system (5 kDa cutoff membrane, Milipore). This concentrated filtrate was designated as crude extract. The crude extract was diluted to below 2 ms $cm^{-1}$, adjusted to pH 8.0, and applied to a HiTrap™ Q XL column (Amersham Pharmacia Biotech, Sweden) that had been equilibrated with 10 mM sodium phosphate buffer. Bound proteins were eluted with a linear increasing NaCl gradient (0–1.0 M). Elution of proteins was monitored at 280 nm. Fractions of 2.0 ml were collected and qualitatively assayed for protease activity.

Fractions containing protease activity from the anion-exchange chromatography were pooled and mixed with 3.4 M $(NH_4)_2SO_4$ in a proportion of 3:2 (v/v, sample: buffer). The sample was applied to a HiPrep™ 16/10 Phenyl FF (high sub) column (Amersham Pharmacia Biotech, Sweden) connected to a FPLC system. The two buffers used were: A, 50 mM sodium phosphate buffer (pH 7.0) with 1 M ammonium sulfate; B, 50 mM sodium phosphate buffer (pH 7.0). Fractions of 0.5 ml were collected and qualitatively assayed for protease activity. After SDS-PAGE analyses, fractions with protease activity and a single band were pooled and stored in −20°C for further analysis.

### SDS-PAGE and N-terminal amino acid sequence analysis

SDS-PAGE was performed with a Mini-PROTEAN III gel system (Bio-Rad, USA), using slab gels, 0.5 mm thick, of 12% polyacrylamide, according to the method of Laemmli (1970). After electrophoresis, the proteins were stained with Coomassie Blue G-250.

The N-terminal amino acid sequence of the purified protease Ac1 was determined on an ABI Procise 491 protein sequencer (Applied Biosystems, USA). The detailed method and protocol were described in a previous report (Yang et al. 2005a).

### Characterization of the purified enzyme

The optimum pH was determined by mixing the purified protease with the barbital sodium-NaOH buffer system (Yang et al. 2005a) at pH values between 3 and 9, and the optimum temperature was determined by incubating the reaction mixture at different temperatures ranging from 35 to 75°C.

The purified protease was assayed against casein in the presence of protease inhibitors and various metal ions (Table 2). Protease activity was measured as described above, after protease had been incubated with inhibitors at room temperature for 10 min.

Proteinous substrates hydrolysis was tested by suspending each of various substrates (Table 3) in 50 mM sodium phosphate buffer (pH 7.0).

### Amplification of the Ac1 gene

*Arthrobotrys conoides* was grown in PL-4 medium on a rotary shaker (150 rpm) at 26°C for 4 days, the mycelium was filtered on a nylon mesh and genomic DNA was

isolated using the DNeasy Plant Mini Kit (Qiagen, German) according to the user's manual.

The encoding gene of Ac1 was amplified according to the method described by Wang et al. (2006). Two degenerate primers were designed according to the conserved sequences of the cuticle-degrading proteases PII, Aoz1 and Mlx from nematophagous fungi *A. oligospora* and *M. microscaphoides* (GenBank accession nos. **X94121**, **AF516146** and **AY841167**, respectively). Sequence for the upstream primer (PP4) was 5′-(A/C)A(A/T)G(A/C)T(G/T)(A/T)(C/T)GAAC(G/C)(G/T)(C/T)CT-3′, and that of the downstream primer (FF2), 5′-TTAAG(C/T)(G/A)(G/T)(A/T/C)(G/T) CC(G/A)TTG(A/T)A-3′. The genomic DNA was used as template, and PCR conditions followed those described in a previous report (Wang et al. 2006).

### Sequencing and analysis

The PCR products were purified from the 1% agarose gel using a DNA fragment purification kit ver 2.0 (Takara, Japan) and subcloned into pGEM–T Vector (Promega, USA). Randomly selected white colonies were purified using the plasmid DNA purification kit (Qiagen, German) and the plasmid DNA was sequenced using an ABI 3730 autosequencer (Perkin-Elmer, USA) with four fluorescent dyes. The sequencing primers were T7 and SP6 universal primers. Sequence data were analyzed using the DNAman software package (Version 5.2.2, Lynnon Biosoft, Canada). Sequence identity was performed using BlastX (http://www.ncbi.nlm.nih.gov/BLAST/). Signal sequence prediction was performed using SignalP (http://www.cbs.dtu.dk/services/SignalP/). N-linked glycosylation sites were predicted by NetNGlyc (http://www.cbs.dtu.dk/services/NetNGlyc/).

### Phylogenetic analysis of serine proteases from nematophagous fungi

The deduced peptide sequences of serine proteases from entomopathogenic and nematophagous fungi were aligned using Clustal X 1.83 (Thompson et al. 1997) and BioEdit softwares (Hall 1999). Manual gap adjustments were made to improve the alignment. Phylogenetic analyses were conducted with the PHYLIP program package (Felsenstein 1991). The data were subjected to Neighbour-Joining method of phylogenetic analysis, and the branch support of the NJ tree was evaluated using bootstrap analysis with 1,000 replications.

## Results

### Purification of extracellular protease

The protease was produced on the third day and reached the highest activity on the sixth day at 26°C. Purification factors and yields at each step are summarized in Table 1. Pigments and partial proteins were removed using an anion-exchange column. The final preparation migrated as a single band of protein on SDS-PAGE, indicating a homogeneous protein with an apparent molecular mass of 35 kDa (Fig. 1).

### Effects of the pH and temperature on enzyme activity

The effects of pH and temperature on the catalytic-activity of Ac1 were very noticeable. The optimum temperature of the enzyme was determined to be 53.2°C. The enzyme

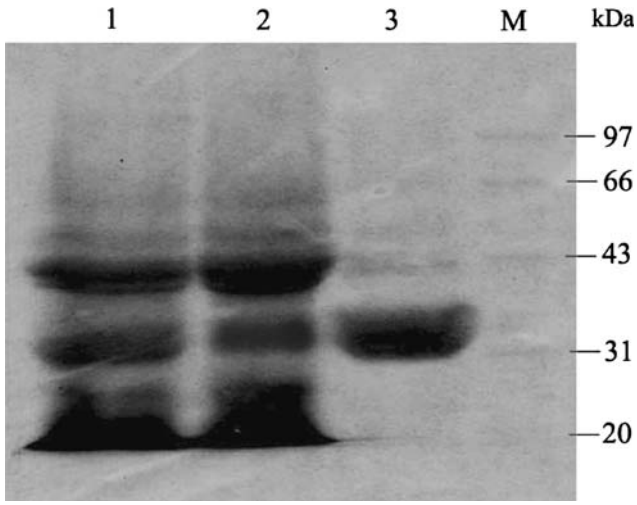


**Fig. 1** SDS-PAGE electrophoresis gel. *Lane 1 and 2* Crude extract. *Lane 3* Purified protease Ac1. *Lane M* protein marker

**Table 1** Purification of the extracellular protease (Ac1) from *A. conoides*

| Purification procedure | Total protein (mg) | Total activity (U)[a] | Specific activity (U mg$^{-1}$) | Purification factor | Yield (%) |
|---|---|---|---|---|---|
| Crude extract | 230.6 | 3043.9 | 13.2 | 1.0 | 100 |
| HiTrap™ Q XL | 168.5 | 2686.3 | 15.9 | 1.3 | 88.3 |
| HiPrep™ 16/10 Phenyl FF | 36.3 | 992.6 | 27.3 | 2.3 | 32.6 |

[a] One unit (U) of protease was defined as the amount of enzyme that hydrolyzed the substrate and produced 1 mg tyrosine in 1 min under the assay conditions

activity was stable at 42–60°C for 20 min incubation, but completely inactivated at temperature over 70°C after 20 min incubation. The purified protease had the maximum hydrolytic activity at pH 7.0. Protease activity increased from pH 4.0 to pH 7.0, and increased from pH 7.0 to pH 9.0.

## Effects of protease inhibitors and metal ions on enzyme activity

The effects of protease inhibitors and various metal ions on the enzyme activity were determined. Only in the presence of PMSF, the enzyme activity was strongly inhibited (Table 2), indicating that Ac1 belongs to the serine-type peptidase group, while metal chelator (EDTA) had no obvious effect on the proteolytic activity. The proteolytic activity of Ac1 was inhibited by 0.5 mM $Cu^{2+}$ (72.6% inhibition) and enhanced by 0.5 mM $Fe^{2+}$ (106%). However, $Mg^{2+}$, $Zn^{2+}$ and $Ca^{2+}$ had only weak effects on the protease.

## Hydrolysis of proteinous substrates

Hydrolytic activity of protease Ac1 was particularly high for degrading casein, moderately for BSA, collagen, denatured collagen and gelatin, and low for nematode cuticle (Table 3).

## Nematicidal activity analysis

The effect of the Ac1 protease on nematode was investigated by in vitro assays as follows: approximately 50 nematodes were added to solutions of either the purified protease Ac1, crude protease extracts, or boiled Ac1, respectively. The mixtures were incubated at 26°C for 12–36 h, and the number of dead nematodes was observed under a light microscope for each treatment. The results are summarized in Table 4. Both the crude enzyme and the purified protease could immobilize the free-living nematode *P. redivivus* and the pine wood nematode *B. xylophilus*. The majority of *P. redivivus* (60–80%) was immobilized after being treated with crude enzyme and purified protease for 24 h, but only 40–50% of *B. xylophilus* were immobilized. Overall, the crude enzyme was more effective than the purified protease. The nematode cuticle of *P. redivivus* was degraded after being treated with purified Ac1 for 24 h (Fig. 2), however the protease Ac1 could not degrade the cuticle of *B. xylophilus*, even after the nematode was dead.

**Table 2** Effects of metal ions and inhibitors on the protease activity of Ac1

| Metal ions and inhibitors | Ions/inhibitors concentration (mM) | Protease relative activity |
|---|---|---|
| Control | – | 100 |
| $Mg^{2+}$ | 0.5 | 97.1 |
| $Cu^{2+}$ | 0.5 | 27.4 |
| $Zn^{2+}$ | 0.5 | 98.2 |
| $Ca^{2+}$ | 0.5 | 95.1 |
| $Fe^{2+}$ | 0.5 | 106 |
| PMSF | 1.0 | 8.17 |
| EDTA | 10 | 100 |

Purified enzyme was incubated with the substrates, metal ions and inhibitors at 53.2°C for 20 min. The maximum proteolytic activity corresponding to 100% was 27.3 U $mg^{-1}$ , for three replicates. The relative activity was presented as the percentage of the maximum proteolytic activity

**Table 3** Hydrolysis of various protein substrates by serine protease Ac1

| Substrate | Relative activity (%) |
|---|---|
| Casein | 100 |
| Gelatin | 21.9 |
| Bovine serum albumin | 37.4 |
| Collagen[a] | 20.7 |
| Denatured collagen[b] | 23.8 |
| Nematode cuticle[c] | 4.1 |

Purified protease was incubated with the different substrates at 53.2°C for 20 min. The maximum proteolytic activity corresponding to 100% was 27.3 U $mg^{-1}$ , for three replicates

[a] Collagen, Purchased from Sigma (Collagen I, C9879)

[b] Denatured collagen, collagen heated at 100°C for 15 min

[c] Fragments of cuticle prepared from the nematode *P. redivivus*. Nematode cuticle was isolated according to the method of Cox et al. (1981)

## Cloning and sequence analysis

A 1297-bp PCR fragment was amplified using the degenerate primers described in Materials and methods. The nucleotide sequence of *Ac1* comprised an open reading frame (ORF), which contained one intron and two exons (Fig. 3). The intron contained 60 nucleotide acid residues and began with GT and ended with AG, which was a common feature of fungal introns and had also been observed in the serine protease gene from *Acremonium chrysogenum* (Isogai et al. 1991). The Ac1 encoding gene had been submitted to GenBank, under accession number **AY859782**.

Comparison of Ac1 (translated amino acid sequence) to fungal proteases revealed that it was typical of fungal serine proteases. It possesses a pre-pro-peptide structure, indicating that it is translated as a precursor polypeptide consisting of 411 amino acids with a calculated molecular mass of 42.6 kDa. It has a signal peptide (21 amino acids) consisting of the initial methionine, a core of eight hydrophobic residues (Leu-Leu-Ala-Ile-Ala-Gly-Leu-Ala), and three hydrophobic residues before a signal peptidase cleavage

**Table 4** Nematicidal activity analysis of protease Ac1 from *A. conoides*

| Samples | Protease activity (U $mg^{-1}$) | *Panagrellus redivivus* (Mortality[a], %) | | | *Bursaphelenchus xylophilus* (Mortality, %) | | |
|---|---|---|---|---|---|---|---|
| | | 12 h | 24 h | 36 h | 12 h | 24 h | 36 h |
| Control[b] | 0 | 10 ± 2 | 15 ± 3 | 30 ± 5 | 2 ± 1 | 5 ± 1 | 5 ± 2 |
| Crude extract | 13.2 | 55 ± 2 | 80 ± 5 | 100 | 30 ± 2 | 50 ± 2 | 60 ± 2 |
| HiTrap™ Q XL | 15.9 | 20 ± 3 | 70 ± 3 | 100 | 30 ± 2 | 50 ± 2 | 55 ± 2 |
| HiPrep™ 16/10 Phenyl FF | 27.3 | 20 ± 3 | 60 ± 5 | 100 | 30 ± 3 | 40 ± 2 | 50 ± 2 |

[a] The proportion of dead nematodes to total nematodes

[b] Protease heated at 100°C for 20 min

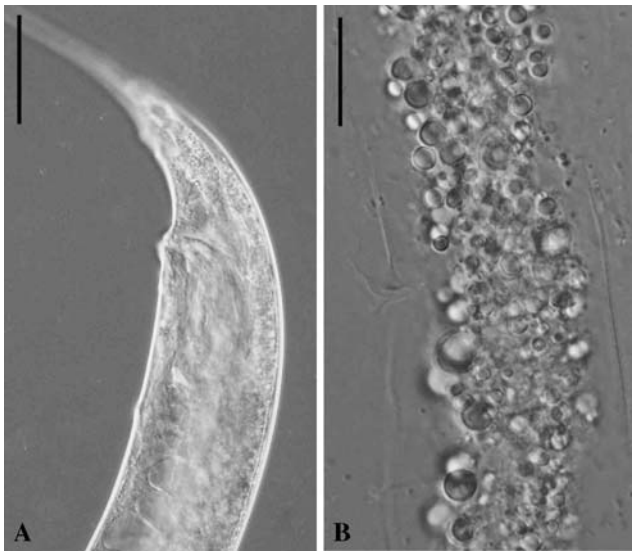


**Fig. 2** Nematode treated with purified protease. **a** Control nematode. **b** Nematode (*Panagrellus redivivus*) treated with protease Ac1. *Scale bar* 100 μm

site (Ala-Phe-Ala). The pro-peptide cleavage site is before the N-terminus of the secreted protein and the final residue of the pro-peptide is an asparagine (N), position 123 in Ac1. The first ten amino acids of the mature protease determined by protein sequencer are AEQTDSTWGL, the same as the predicted N-terminus sequence of Ac1. The mature peptide consists of 288 amino acids with a calculated molecular mass of 29.7 kDa.

The primary sequences of the fungal serine proteases were aligned using the DNAman software package (Fig. 4). The deduced peptide sequence of the Ac1 showed 88.2, 81.0, 44.1, 42.2, 42.2 and 42.2% sequence identity, respectively, to PII (Åhman et al. 1996), Mlx (Wang et al. 2006), VCP1 (Morton et al. 2003), Ver112 (Yang et al. 2005b), PIP (Bonants et al. 1995) and Prk (Gunkel and Gassen 1989). These proteases shared the conservation of the aspartic acid ($Asp_{164}$)-histidine ($His_{200}$)-serine ($Ser_{353}$) (in Ac1) catalytic triad. The two blocks of side-chains that form the sides of the substrate-binding $S_1$ pocket in subtilisin occur in regions of high similarity and consist of $Ser_{259}$ $Leu_{260}Gly_{261}$ and $Ala_{285}Ala_{286}Gly_{287}$, respectively, in Ac1. Furthermore, the highly conserved $Asn_{288}$ (in Ac1) is important in subtilisin for the stabilization of the reaction intermediate formed during proteolysis (Kraut 1977).

The deduced primary sequence of the mature protease Ac1 contains four potential N-linked glycosylation sites ($Asn_{178}$, $Asn_{252}$, $Asn_{394}$ and $Asn_{408}$), which follows the general rule of Asn-Xaa-Ser/Thr, where X is any residue except perhaps aspartate, glutamic acid and proline (Mononen and Karjalainen 1984). Similarly, PII and Aoz1 contain two potential N-linked glycosylation sites ($Asn_{177}$ and $Asn_{251}$), but Mlx ($Asn_{175}$) contains only one site. However, those three proteases (Ver112, PIP and VCP1) from endoparasitic fungi lack the potential N-linked glycosylation site found in Ac1.

## Phylogenetic analysis of serine proteases from nematophagous fungi

Phylogenetic tree (Fig. 5) was constructed based on the deduced peptide sequences from nematophagous, entomopathogenic and non-pathogenic fungi by the PHYLIP program package. From this tree (Fig. 5), the pathogencity-

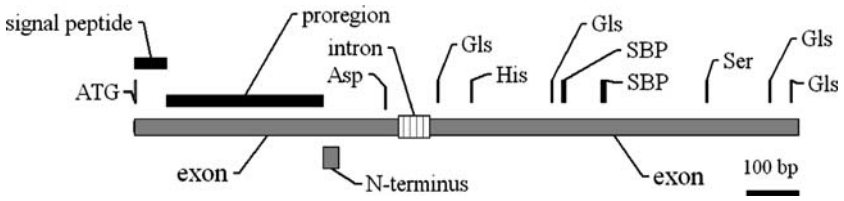


**Fig. 3** Diagram of Ac1 gene showing the presence of intron/exons and basic features. The length of the sequence is 1,297 bp. ATG: Start codon. Asp, His and Ser: Active sites. Gls: N-linked glycosylation sites. *SBP* Substrate-binding S1 pocket in Ac1

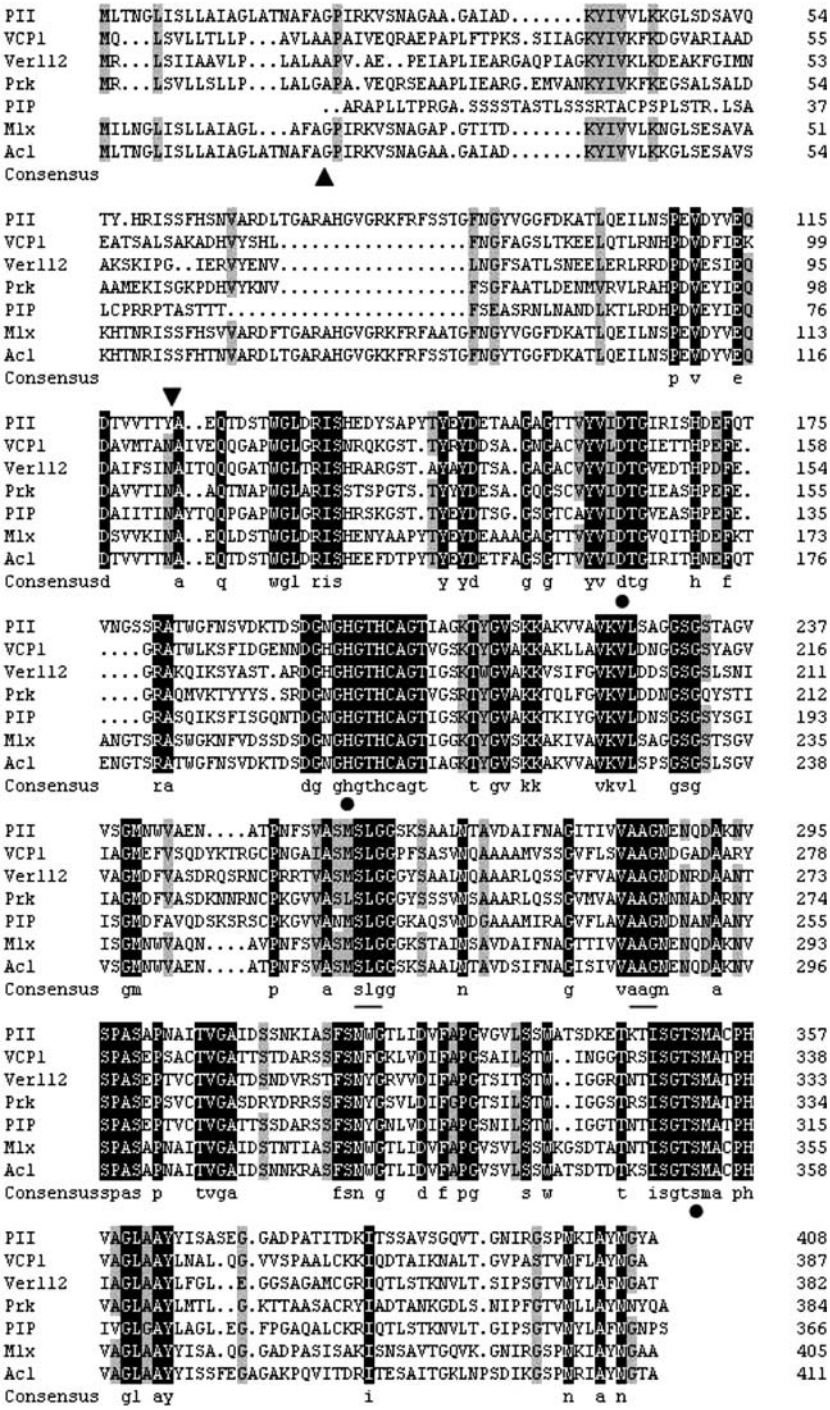


**Fig. 4** Alignment of cuticle-degrading proteases amino acid sequences from *A. conoides* (Ac1), *A. oligospora* (PII), *M. microscaphoides* (Mlx), *T. album* (Prk), *P. lilacinus* (PIP), *L. psalliotae* (Ver112) and *P. chlamydosporia* (VCP1). The GenBank accession nos. of Ac1, PII, Mlx, Prk, PIP, Ver112 and VCP1 are **AAX54903**, **CAA63841**, **AAW21809**, **P06873**, **AAA91584**, **AAU01968** and **CAD20578**, respectively. Areas shaded in *black* are conserved regions (100% similarity), areas shaded in *grey* are high degree homology (more than 75% similarity) and *unshaded* areas are regions of variability between the proteases. *Filled triangle* indicates signal peptidase cleavage site (in Ac1). *Filled inverted triangle* indicates putative proregion cleavage site (in Ac1). *Filled circle* indicates the aspartic acid ($Asp_{164}$)-histidine ($His_{200}$)-serine ($Ser_{353}$) (in Ac1) catalytic triad. The *underlined* region is the substrate-binding $S_1$ pocket in subtilisin

related serine proteases from nematophagous and entomopathogenic fungi were found to have evolved from an ancestor. Among them, four proteases (PII, Aoz1, Ac1 and Mlx) identified from nematode-trapping fungi formed a clade. Another clade consisted of two subclades, proteases (Ver112, VCP1 and PIP) identified from nematode-parasitic fungi clustered together with Pr1 and PrA, which were isolated from entomopathogenic fungi *Beauveria bassiana* (Joshi et al. 1995) and *Metarhizium anisopliae* (St Leger et al. 1992). However, Protease K (Prk) from *Tritirachium album* (Gunkel and Gassen 1989) formed a subclade and diverged from the proteases isolated from parasitic fungi.

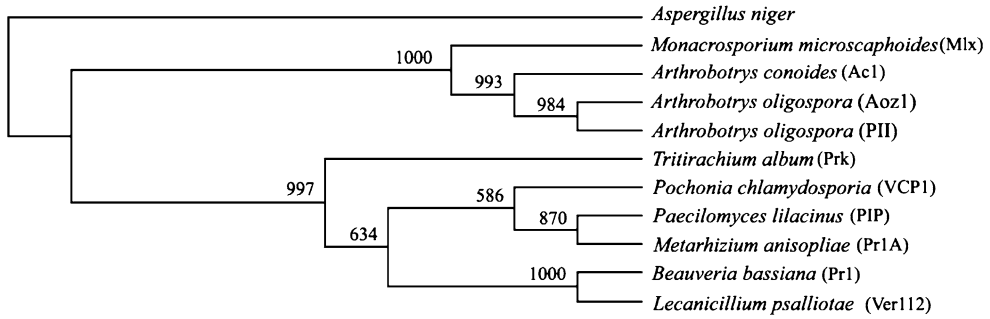


**Fig. 5** Phylogenetic tree showing the relationship between Ac1 and other fungal subtilases. The GenBank accession numbers of proteases Pr1 (*B. bassiana*) and PrA (*M. anisopliae*) are AAK70804 and CAB64346, respectively. Those of the other proteases were described in Fig. 4. *Aspergillus niger* (accession number: AAA32703) was used as outgroup

## Discussion

In this report, an extracellular serine protease was identified for the first time from the nematode-trapping fungi *A. conoides*. Our results further confirmed the roles of serine proteases in the pathogenesis against nematodes and extended the knowledge about pathogencity-related serine proteases from nematode-trapping fungi.

Serine proteases including Ac1 from nematophagous fungi can degrade a broad range of substrates including casein, gelatin, bovine serum albumin, collagen, and nematode cuticle (Tunlid et al. 1994; Yang et al. 2005a; Wang et al. 2006). However, Ac1 exhibits several properties different from previously reported proteases. Three proteases Ac1, Aoz1 and Mlx from closely species of nematode-trapping fungi (*A. conoides*, *A. oligospora* and *M. microscaphoides*) showed optimum temperatures at 53.2, 45 and 65°C, respectively. Moreover, these proteases showed pH ranges from pH 6 to pH 9. The optimum pH range of Aoz1 is pH 6–8, PII is pH 7–9, Mlx is pH 9 and Ac1 is pH 7 (Tunlid et al. 1994; Zhao et al. 2004; Wang et al. 2006). Compared to PII, Ac1 appears to have a significantly higher activity against gelatin, bovine serum albumin, and non-denatured collagen. These differences in biochemical properties may be related to substrate preference and to their differential pathogencity against different hosts (nematodes) (Morton et al. 2003).

These pathogenic serine proteases shared a high degree of similarity (Fig. 4) (>81% on aa level) to those from closely related species of nematode-trapping fungi. This result suggests that these proteases may play a similar role during infection. Based on the phylogenetic analysis (Fig. 5), these pathogenicity-related serine proteases likely evolved from a common ancentor. Among them, four proteases (PII, Aoz1, Ac1 and Mlx) from nematode-trapping fungi formed a clade, which shared extensive similarities (Fig. 4). Another clade consisted of two subclades: proteases from nematode-parasitic fungi and entomopathogenic fungi formed a subclade, and Prk from non-pathogenic fungus *T. album* (Gunkel and Gassen 1989) formed a single subclade. Therefore, these pathogenicity-related proteases may have evolved and adapted to different environment.

*Arthrobotrys conoides* infects nematodes by producing adhesive networks (Saxena et al. 1987). In this study, we found *P. redivivus* is more effective at inducing trap production than *B. xylophilus*, and *A. conoides* showed a higher ability to infect *P. redivivus*. These differences may be the result of substrate preferences of Ac1 to the proteinaceous components of different nematodes (*P. redivivus* and *B. xylophilus*). Moreover, Ac1 is more effective at immobilizing *P. redivivus* than *B. xylophilus* (Table 4). And, the cuticle of *P. redivivus* can be effectively degraded by Ac1 (Table 3, Fig. 2). However, the cuticle of *B. xylophilus* is hard to be degraded, suggesting some differences in the chemical components of cuticles between *P. redivivus* and *B. xylophilus*. In fact, the nematode cuticle is a complex structure. Its surface may be covered with a coat of glycoproteins and other surface-associated proteins, or rarely with an additional sheath. These components are important in for recognition with plant hosts and microbial antagonists (Cox et al. 1981; Bird and Bird 1991). Additionally, the structure and physical properties of nematode cuticles vary with life stages (Abrantes and Curtis 2002). Therefore, host preferences of nematophagous fungi (e.g. *A. conoides*) to saprophytic and parasitic nematodes may be important for exploring nematophagous fungi as biocontrol agents against different nematode diseases and for improving the efficacy of nematophagous fungi through genetic engineering (Åhman et al. 2002).

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