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Purification and Cloning of an Extracellular Serine Protease from the Nematode-Trapping Fungus *Monacrosporium cystosporium*

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An extracellular protease (Mc1) was isolated from the nematode-trapping fungus *Monacrosporium cystosporium* by gel filtration, anion-exchange, and hydrophobic interaction chromatographies. This protease had a molecular mass of approximately 38 kDa and displayed an optimal activity at pH 7–9 and 56°C (over 30 min). Its proteolytic activity was highly sensitive to the serine protease inhibitor PMSF (phenylmethylsulfonylfluoride, 0.1 mM), indicating that it belonged to the serine-type peptidase group. The Michaelis constant (K_m) and V_{max} for substrate *N*-Suc-Ala-Ala-Pro-Phe-*p*NA were 1.67×10^{-4} M and 0.6071 OD_{410} per 30 s, respectively. This protease could degrade a broad range of substrates including casein, gelatin, BSA (bovine serum albumin), and nematode cuticle. Moreover, the enzyme could immobilize the free-living nematode *Panagrellus redivivus* and the pine wood nematode *Bursaphelenchus xylophilus*, suggesting that it might play a role in infection against nematodes. The encoding gene of Mc1 was composed of one intron and two exons, coding for a polypeptide of 405 amino acid residues. The deduced amino acid sequence of Mc1 showed 61.4–91.9% identity to serine proteases from other nematode-trapping fungi. Our results identified that Mc1 possessed biochemical properties including optimal reaction condition and substrate preference that are different from previously identified serine proteases.

Keywords: *Monacrosporium cystosporium*, protease, purification, gene cloning, nematicidal activity

A large number of species belonging to fungi, bacteria, viruses, insects, mites, and some invertebrates have been found capable of invading or preying on nematodes [16, 17]. Among these organisms, nematophagous fungi, one of the natural enemies of nematodes, have been proposed as biological agents to control harmful nematodes because of their unique ability to infect and kill the nematodes [14, 16, 27].

The nematophagous fungi comprise more than 200 taxonomically diverse species that all share the ability to infect and kill living nematodes [14]. According to their pathogenic mechanisms, nematophagous fungi are traditionally grouped into three categories: nematode-trapping fungi, parasitic fungi, and toxic fungi [11, 14, 16]. Among them, nematode-trapping fungi can form different nematode-trapping devices for capturing nematodes [14, 27]. This group is traditionally classified into three genera, *Arthrobotrys* Corda, *Dactylella* Grove, and *Monoacrosporium* Oudem, based on their morphologic properties [18].

The nematode cuticle is a complex structure that is important in motility, maintenance of morphological integrity, and protection against environment stresses and potential pathogens. As with other pathogens, the nematode-trapping fungi typically enter into the host through a combination of enzyme degradation and mechanical pressure [13, 21, 27]. At present, the detailed molecular pathogenic mechanisms against nematodes by nematode-trapping fungi have not yet been fully elucidated. However, increasing evidence has shown that extracellular hydrolytic enzymes including proteases, collagenase, and chitinase may be involved in the penetration of nematode-cuticle and host-cell digestion [1, 13, 27]. Ever since Tunlid *et al.* [21] isolated a serine protease (PII) from the nematode-trapping fungus *Arthrobotrys oligospora*, more than 5 pathogenicity related proteases have been identified from nematode-trapping fungi [10, 22, 23, 25, 26, 28]. At present, little information is known about the extracellular enzymes involved in the infection process by nematode-trapping fungi in the genus *Monacrosporium* Oudem. Such studies should help identify more pathogenicity related proteases from different nematode-trapping fungi in order to elucidate the pathogenesis of nematophagous fungi against nematodes.

In this report, we describe the purification and biochemical properties of an extracellular serine protease (Mc1) from the nematode-trapping fungus *M. cystosporium*. Furthermore,

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we cloned the encoding gene of Mc1 and compared its sequence with other cuticle-degrading serine proteases isolated from different nematode-trapping fungi.

MATERIALS AND METHODS

Organisms and Growth Conditions

The nematode-trapping fungus *M. cystosporium* (CGMCC 1309) used in this study was incubated on potato dextrose agar (PDA) at 26°C. The PL-4 liquid medium for protease production is described in our previous report [24]. *Escherichia coli* strain DH5α was used in all DNA manipulations and was grown in Luria-Bertani medium at 37°C.

Nematodes *Panagrellus redivivus* and *Bursaphelenchus xylophilus* were cultured and maintained according to the method described by Dong *et al.* [5]. The nematodes were separated and washed thoroughly with 50 mM sodium phosphate buffer (pH 7.0) before being used in the assays.

Protease Activity Analysis

Protease activity was determined by a caseinolytic method described in our previous report [22]. Protein concentration was determined using the method of Bradford [3] with BSA as a standard. One unit (U) of protease activity was defined as the amount of enzyme that hydrolyzed the substrate and produced 1 μg of tyrosine in 1 min under the assay conditions.

Protease Purification

Monacrosporium cystosporium was cultured in PL-4 liquid medium for 7 days at 26°C. The culture filtrate (2 l) was collected by vacuum filtration and protease in the filtrate was concentrated using ammonium sulfate (56%, w/v). The sample was applied to a HiLoad 16/60 Superdex column (Amersham, Sweden) equilibrated with 50 mM sodium phosphate (pH 7.0) containing 0.15 M sodium chloride and eluted with the same buffer at 1 ml/min. Fractions containing protease activity were pooled and balanced with 10 mM sodium phosphate (pH 7.5). The sample was applied to a Resource Q column (Amersham, Sweden) equilibrated with 10 mM sodium phosphate (pH 7.5) and the bound proteins were eluted with 10 mM sodium phosphate (pH 7.5) containing 0.5 M sodium chloride at 1 ml/min. Fractions containing protease activity from the Resource Q column were pooled and mixed with 3.4 M ammonium sulfate 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, Sweden) equilibrated with 50 mM sodium phosphate (pH 7.0) containing 1 M ammonium sulfate, and then eluted with 50 mM sodium phosphate (pH 7.0) at 2 ml/min. Fractions of 0.5 ml were collected and qualitatively assayed for protease activity. After SDS-PAGE analyses, fractions with protease activity 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, U.S.A.), using slab gels (0.5 mm thick, 12% polyacrylamide) according to the method of Laemmli [9]. The proteins were stained with Coomassie Blue G-250. The N-terminal amino acid sequence of the purified protease Mc1 was determined according to a previously described method [22].

Effects of Temperature and pH on Enzyme Activity

The optimum pH and optimum temperature for enzyme activity as well as the stabilities of the enzyme at various pH and temperatures were determined as described in our previous report [24]. Briefly, the optimum pH was determined by mixing the purified protease with the Britton-Robinson buffer system 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.

Effects of Metal Ions and Protease Inhibitors on the Enzyme Activity

The purified protease was mixed with its substrate (casein), with different metal ion solutions (0.5 mM) or protease inhibitors (EDTA and PMSF) added respectively. These mixtures were incubated at optimum reaction conditions for 30 min. Their protease activities were then measured according to the method described by Wang *et al.* [22]. The experiment was repeated three times.

Hydrolysis of Protein and Peptide Substrates

The purified protease was incubated with different protein and peptide substrates at pH 8 and 56°C for 30 min and the protease activity was quantitatively assayed. Nematode cuticle was extracted according to the method of Cox *et al.* [4].

K_m and V_{max} values for *N*-Suc-Ala-Ala-Pro-Phe-*p*NA were determined using Lineweaver-Burk plots, and the reaction rate (V) was defined as the increasing of OD_{410} in 30 s under the assay conditions.

Amplification of the Mc1 Gene

Monacrosporium cystosporium was grown in PL-4 medium on a rotary shaker (150 rpm) at 26°C for 4 d. The mycelium was filtered on a nylon mesh and genomic DNA was isolated using the DNeasy Plant Mini Kit (Qiagen, Germany) according to the user's manual. The encoding gene of Mc1 was cloned according to our previous report [22], and two degenerate primers PP4 [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 FF2 [5'-TTAAG(C/T)(G/A) (G/T)(A/T/C) (G/T)CC(G/A)TTG(A/T)A-3'] were designed on the basis of the gene sequences of serine proteases from the nematophagous fungi *A. oligospora* (PII and Aoz1) [1, 28], *Monacrosporium microscaphoides* [22], and *Lecanicillium psalliotae* [24]. The GenBank accession numbers of these serine protease genes are X94121, AF516146, AY841167, and AY692148, respectively. The genomic DNA was used as a template, and the PCR conditions followed those described in a previous report [22].

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 pMD-18T Vector (Takara, Japan). Randomly selected white colonies were purified using the plasmid DNA purification kit (Qiagen, Germany) and the plasmid DNA was sequenced using an ABI 3730 automated sequencer (Perkin-Elmer, U.S.A.) with four fluorescent dyes. Sequence data were analyzed using the DNAman software package (Version 5.2.2; Lynnon Biosoft, Canada). Homology search 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/).

Table 1. Summary of the purification procedures of protease Mc1.

Purification procedure	Volume (ml)	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification factor	Yield (%)
Culture filtrate	2,000	424.0	126,355.6	298.0	1.0	100
Crude enzyme[a]	28	131.4	48,946.5	372.5	1.25	38.8
HiLoad 16/60 Superdex	160	105.3	42,678.1	405.3	1.36	33.8
Resource Q	20	6.26	8,340.0	1,332.7	4.47	6.6
HiPrep 16/10 Phenyl FF	3.3	0.76	3,121.1	4,060.6	13.6	2.5

[a]Protease was concentrated using ammonium sulfate.

Phylogenetic Analysis of Serine Proteases from Nematophagous Fungi

The deduced peptide sequences of serine proteases from nematophagous fungi were aligned using ClustalX 1.83 [20]. Manual gap adjustments were made to improve the alignment. Phylogenetic analyses were conducted with the Mega program package [19]. The data were subjected to the neighbour-joining method of phylogenetic analysis, and the branch support of the NJ tree was evaluated using bootstrap analysis with 1,000 replications.

RESULTS

Protease Production and Purification

Cculture filtrates (2 l) were harvested by vacuum filtration and the protease was purified by chromatography. The purification factors and recoveries at each step are summarized in Table 1. The culture filtrate was concentrated and the protease Mc1 was purified by gel filtration, anion-exchange, and hydrophobic interaction chromatographies. About 2.5% protease activity was recovered with a 13.6-fold purification (Table 1). The purified protease showed as a single protein band on the 12% Coomassie Brilliant Blue R-250 stained gel. The molecular mass of the purified Mc1 was estimated to be 38 kDa by SDS-PAGE (Fig. 1).

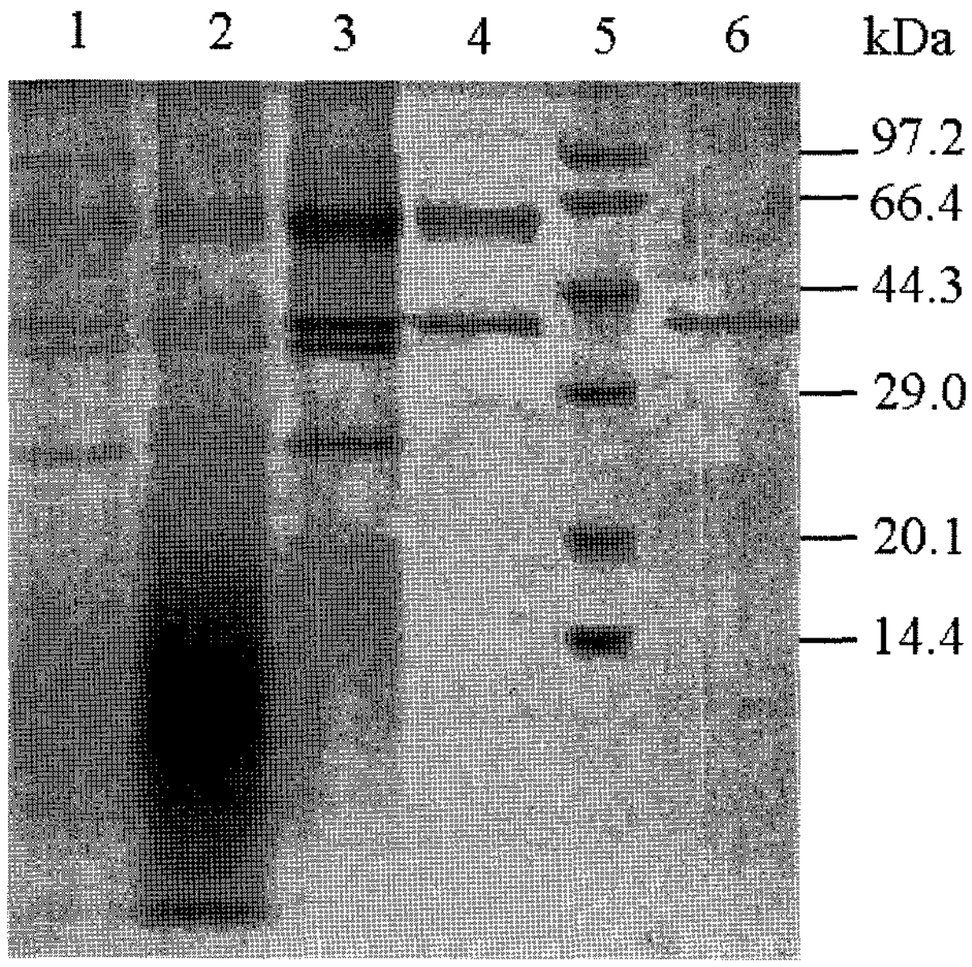

Fig. 1. SDS-PAGE electrophoresis gel.
Lane 1, culture filtrate; lane 2, crude enzyme (protein was concentrated using ammonium sulfate); lane 3, fraction (elution) from the HiLoad 16/60 Superdex column (gel filtrate); lane 4, fraction (elution) from the Resource Q column (anion-exchange); lane 5, protein marker; lane 6, purified Mc1 from the HiPrep 16/10 Phenyl FF (hydrophobic interaction chromatography, HIC).

Effects of the pH and Temperature on Enzyme Activity

The optimum reaction temperature for Mc1 was 56°C, and the enzyme activity was stable when the temperature was below 35°C. However, the enzyme was inactivated at temperatures over 70°C and with 30 min incubation. The protease kept the highest activity between pH 7.0–9.0. Protease activity increased from pH 3.0 to 7.0, and decreased from pH 9.0 to 12.0.

Effects of Protease Inhibitors and Metal Ions

The effects of protease inhibitors and various metal ions on the enzyme activity were determined. The enzyme activity was strongly inhibited by PMSF (Table 2). In contrast, the metal chelator (EDTA) had weak effect on the proteolytic activity (8.4% inhibition, 1 mM). The proteolytic activity of Mc1 was inhibited weakly by 0.5 mM Cu^{2+} (11.4% inhibition) and enhanced by 0.5 mM Ca^{2+} (114.3%). However, Mg^{2+}, Zn^{2+}, and Fe^{2+} had only weak effects on the protease.

Table 2. Effect of metal ions and inhibitor on the enzyme activity.

Metal ions and inhibitors	Ions/inhibitors concentration	Relative activity (%)
Control[a]	–	100
Mg^{2+}	0.5 mM	104±1.0
Cu^{2+}	0.5 mM	88.6±0.5
Zn^{2+}	0.5 mM	106.5±1.0
Ca^{2+}	0.5 mM	114.3±2.0
Fe^{2+}	0.5 mM	102±0.5
PMSF	0.1 mM	0
EDTA[b]	1 mM	91.6±0.5
EDTA	10 mM	71.6±1.0

[a]50 mM sodium phosphate (pH 7.0).
[b]Ethylene diamine tetraacetic acid.

Table 3. Hydrolysis of protein and peptide substrates by protease Mc1.

Protein	Relative activity (%)	Peptide	Relative activity (%)
Casein	100	Suc-Ala-Ala-Pro-Phe-pNA	100
Gelatin	17.7	Suc-Ala-Ala-Pro-Leu-pNA	63
BSA	81.8	Suc-Gly-Gly-Phe-pNA	0.8
Collagen	3.8		
Denatured collagen	23.5		
Nematode cuticle	8.1		

Hydrolysis of Various Protein Substrates

The hydrolytic activity of protease Mc1 was particularly high for degrading casein and BSA (bovine serum albumin), moderate for denatured collagen and gelatin, and low for collagen and nematode cuticle (Table 3).

Moreover, Mc1 showed high activity for degrading Suc-Ala-Ala-Pro-Phe-*p*NA (substrate for chymotrypsin), moderately for Suc-Ala-Ala-Pro-Leu-*p*NA (substrate for elastase), and very low for Suc-Gly-Gly-Phe-*p*NA (substrate for chymotrypsin) (Table 3). The Michaelis constant (K_m) and V_{max} for *N*-Suc-Ala-Ala-Pro-Phe-*p*NA were 1.67×10^{-4} M and 0.6071 OD_{410} per 30 s, respectively.

Nematicidal Activity Analysis

The effect of the Mc1 on nematodes was investigated by *in vitro* assays as follows: approximately 50 nematodes were added to solutions of the purified protease Mc1, crude protease extracts, and boiled Mc1, 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* (70–80%) was immobilized after being treated with the crude and the purified proteases for 24 h, but only 50–60% of *B. xylophilus* were immobilized.

Cloning and Sequence Analysis of the Protease Mc1

A 1,271-bp PCR fragment was amplified using the degenerate primers described in Materials and Methods. The nucleotide sequence of *Mc1* comprised an ORF (open reading frame), which contained one intron and two exons (Fig. 2). The intron contained 51 nucleotides 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* [7]. The Mc1-encoding gene has been submitted to GenBank, under the accession number AY859780.

Comparison of Mc1 (translated amino acid sequence) to fungal proteases revealed that it was typical of fungal serine proteases. It possesses a prepropeptide structure, indicating that it is translated as a precursor polypeptide consisting of 405 amino acids with a calculated molecular mass of 41.1 kDa (Fig. 2). It has a signal peptide (18 amino acids) consisting of the initial methionine and a core of ten hydrophobic residues (Leu-Leu-Ala-Ile-Ala-Gly-Leu-Ala-Phe-Ala). The propeptide cleavage site is before the N-terminus of the secreted protein and the final residue of the propeptide is an asparagine (N), position 120 in Mc1. The first ten amino acids of the mature protease determined by protein sequencing are AEQLGSTWGL, the same as the predicted N-terminus sequence of Mc1. The mature peptide consists of 285 amino acids with a calculated molecular mass of 28.6 kDa.

The primary sequences of serine proteases from nematode-trapping fungi and protease K (PrK) from *Tritirachium album* were aligned using the DNAman software package (Fig. 3). The deduced peptide sequence of the Mc1 showed 91.9%, 84.4%, 81%, 80.7%, 66.4%, and 43.9% sequence identity, respectively, to Mlx [22], PII [1], Ac1 [25], Aoz1 [28], Dv1 [26], and PrK [6]. These proteases shared the conservation of the aspartic acid (Asp_{161})-histidine (His_{197})-serine (Ser_{350}) (in Mc1) catalytic triad (Fig. 3). The two blocks of side-chains that form the sides of the substrate-binding

Table 4. Nematicidal activity analysis of protease Mc1.

Sample	Specific activity (U/mg)	*P. redivivus* (Mortality[a], %)			*B. xylophilus* (Mortality, %)		
		12 h	24 h	36 h	12 h	24 h	36 h
Control[a]	0	0–10	10–15	30–40	2–5	5–10	5–10
Culture filtrate	298.0	20–30	30–40	80–100	10–20	20–30	20–30
Crude enzyme	372.5	50–60	80–90	100	20–30	40–50	40–50
Gel filtration	405.3	20–30	30–50	90–100	20–30	40–50	40–50
Anion-exchange	1,332.7	30–40	40–60	100	30–40	50–60	50–60
HIC	4,060.6	40–60	70–80	100	30–40	50–60	50–60

[a]Boiled purified enzyme used as control.

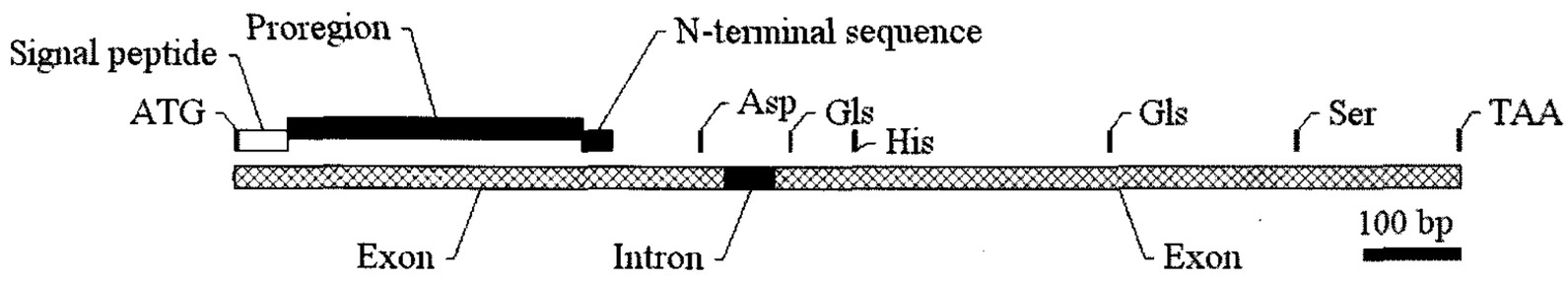

Fig. 2. Diagram of the *Mc1* gene showing the presence of intron/exons and basic features.
The length of the sequence is 1,271 bp. ATG, start codon; Asp, His, and Ser, active sites; Gls, predicted N-linked glycosylation sites in Mc1.

S_1 pocket in subtilisin occur in regions of high similarity and consist of Ser_{256} $Leu_{257}Gly_{258}$ and $Ala_{282}Ala_{283}Gly_{284}$, respectively, in Mc1. Furthermore, the highly conserved Asn_{285} (in Mc1) is important in subtilisin for the stabilization of the reaction intermediate formed during proteolysis [8].

The deduced primary sequence of the mature protease Mc1 contains two potential N-linked glycosylation sites (Asn_{175} and Asn_{285}), which follows the general rule of Asn-Xaa-Ser/Thr, where X is any residue except perhaps aspartate, glutamic acid, and proline [12].

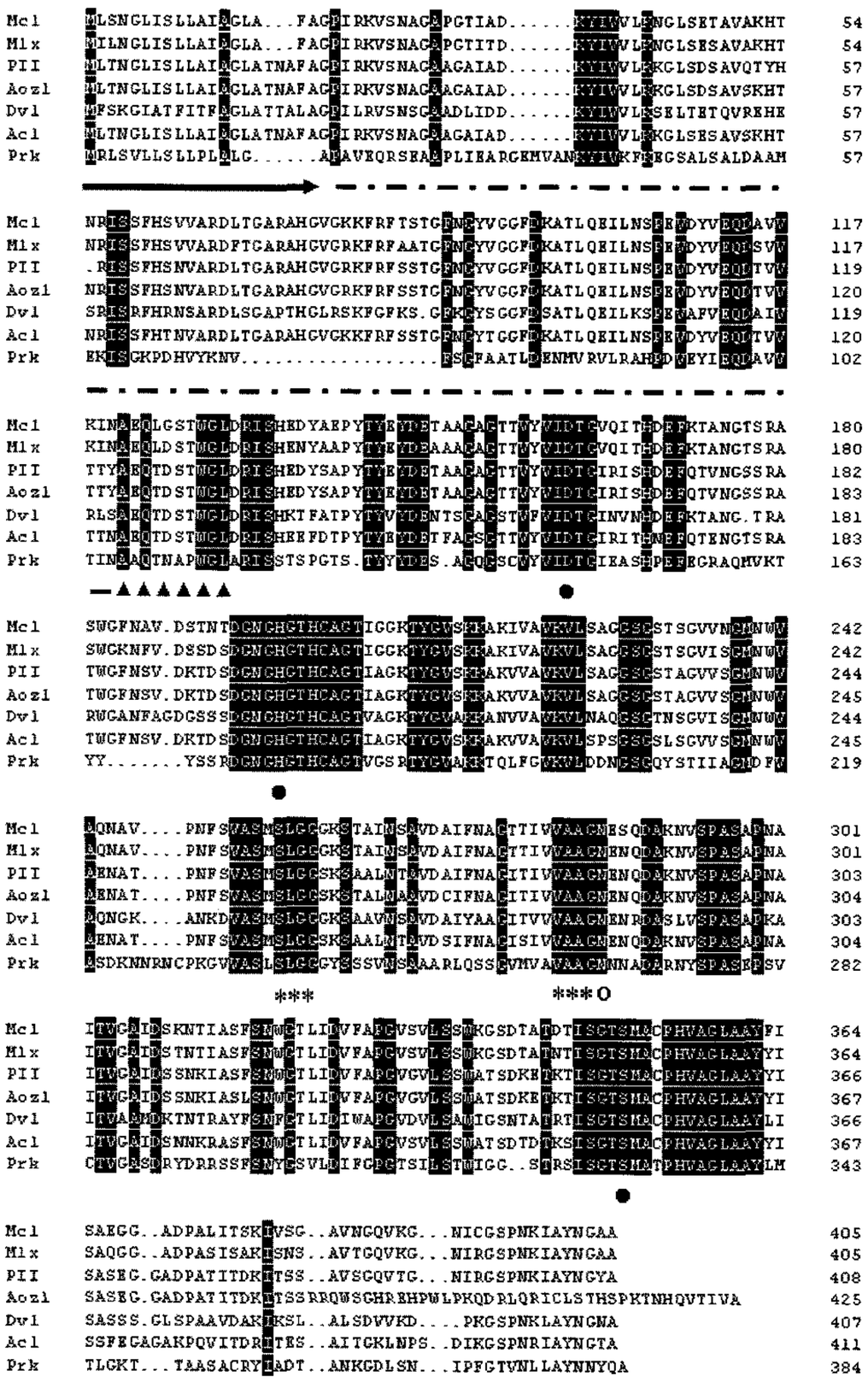

Fig. 3. Alignment of deduced peptide sequences from different fungi.
The GenBank accession numbers of proteases Mc1, Mlx, PII, Aoz1, Dv1, Ac1, and Prk are AAX54901, AAW21809, CAA63841, AAM93666, ABF72192, AAX54903, and CAA32820, respectively. Areas shaded in black are conserved regions (100% similarity), and unshaded areas are regions of variability between these proteases. Signal peptide sequences are marked with an arrow, and propeptides are marked on the discontinuous line. ▲▲▲ indicates the N-terminal sequences of mature peptides. ● indicates the aspartate (Asp_{161})-histidine (His_{197})-serine (Ser_{350}) (in Mc1) catalytic triad. *** indicates the substrate-binding S1 pocket in subtilisin. ○ indicates the oxyanion hole.

Phylogenetic Analysis of Serine Proteases from Nematophagous Fungi

A phylogenetic tree (Fig. 4) was constructed based on the deduced peptide sequences from nematophagous and entomopathogenic fungi by the Mega program package [19]. From this tree (Fig. 4), the pathogenicity related serine proteases from nematophagous and entomopathogenic fungi were found to have evolved from a common ancestor. Among them, six proteases (Mc1, Dv1, PII, Aoz1, Ac1, and Mlx) identified from nematode-trapping fungi formed a clade. Another clade consisted of proteases identified from nematode-parasitic and entomopathogenic fungi. From this tree, these nematode-trapping fungi were clustered consistent with their taxonomic properties.

Discussion

In this study, an extracelluar serine protease, Mc1, was identified and characterized from the nematode-trapping fungus *M. cystosporium*. The encoding gene was cloned and translated as a prepropeptide structure (Fig. 2). This protein was found to have extensive similarities to serine proteases from other nematode-trapping fungi (Fig. 3). Although Mc1 shared common properties with serine proteases from other nematode-trapping fungi, it also showed some novel properties (Table 5). All these proteases are highly sensitive to the serine protease inhibitor PMSF, have similar molecular masses ranging from 30 to 39 kDa, and degrade a similar range of substrates including casein, gelatin, BSA, and nematode cuticle [10, 22, 23, 25, 26, 28]. These results suggest that these proteases play a similar role in infection against nematodes.

Mc1 was highly sensitive to the protease inhibitor PMSF (Table 2), consistent with enzymes in the serine-type peptidases. EDTA also showed weak effect on the

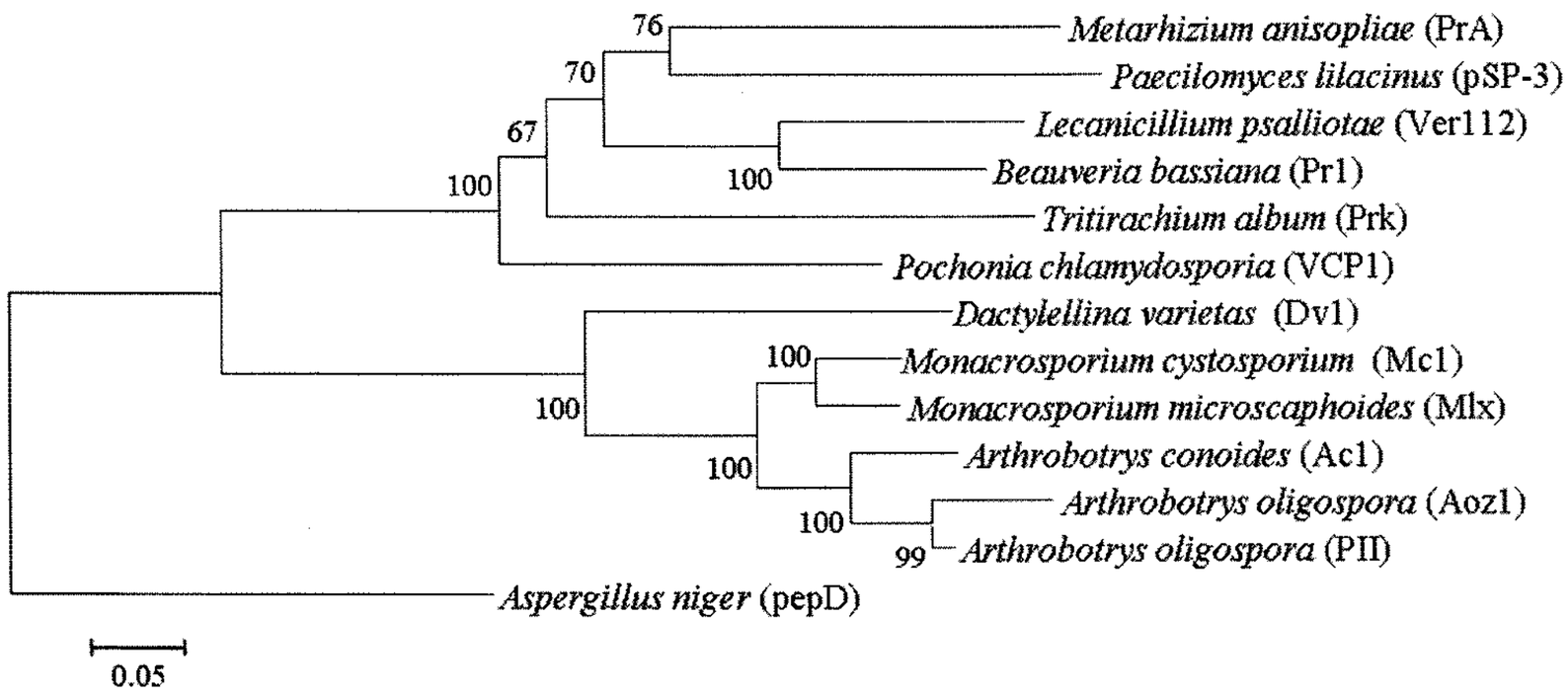

Fig. 4. Phylogenetic tree showing the relationship between Mc1 and other fungal subtilases.
The GenBank accession numbers of proteases Pr1, PrA, Ver112, pSP-3, and VCP1 are AAK70804, CAB64346, AAU01968, AAA91584, and CAD20578, respectively. Those of the other proteases are described in Fig. 3. *Aspergillus niger* (Accession No. AAA32703) was used as outgroup.

proteolytic activity owing to its ability to chelate Ca^{2+}, which plays a role in maintaining the structural stability of serine protease [2]. Moreover, Ca^{2+} can enhance the hydrolytic activity of Mc1 (Table 2).

Different from serine proteases PII, Aoz1, Ac1, and Dv1 from nematode-trapping fungi, Mc1 showed high degrading activity against BSA, similar to that of protease Mlx from *M. microscaphoides* [22]. Mc1 showed extensive sequence similarity (91.9) to Mlx, which suggested the Mc1 is the homolog protease from different species of *Monacrosporium*. Moreover, Mc1 showed a high hydrolytic activity against Suc-Ala-Ala-Pro-Phe-*p*NA (substrate for chymotrypsin), similar to the ability of protease VCP1 from endoparasitic fungus *Verticillium chlamydosporium* [15]. However, the Michaelis-Menten constant (K_m) of VCP1 and Mc1 for Suc-Ala-Ala-Pro-Phe-*p*NA were slightly different, at 4.26×10^{-5} M and 1.67×10^{-4} M, respectively. The results suggest that VCP1 is more effective than Mc1 in degrading polypeptide Suc-Ala-Ala-Pro-Phe-*p*NA. In contrast, Mc1 showed a low degrading activity against another substrate of chymotrypsin Suc-Gly-Gly-Phe-*p*NA, similar to that of PII [21].

The deduced primary sequence of the mature protease Mc1 showed 61.4–91.9% amino acid identity to serine proteases from other nematode-trapping fungi (Fig. 3). These proteases typically have optimal pH ranging from 7.0 to 8.0. However, proteases Mlx and Ds1 showed a greater optimal pH range. Moreover, the optimal reaction temperature was also higher for Dv1 and Mc1, at about 65°C.

Mc1 contains two potential N-linked glycosylation sites (Asn_{175} and Asn_{285}), similar to PII and Aoz1. In contrast, Mlx and Dv1 contains only one potential N-linked glycosylation site each, whereas Ac1 contains four potential N-linked glycosylation sites. The number of glycosylation sites contributes to their differences in molecular masses of the mature proteins, from 30 kDa to 39 kDa [21, 25, 26].

The results in Table 4 show that both the crude extract and the purified protease from *M. cystosporium* had obvious nematicidal effects on *P. redivivus* and *B. xylophilus*. These results suggest that the nematode-trapping fungus *M. cystosporium* may be a potential biocontrol agent against nematodes. Moreover, Mc1 is more effective at immobilizing *P. redivivus* than *B. xylophilus*, consistent with a previous report [25]. These preferences may be the result of substrate preferences of Mc1 for the proteinaceous components of different nematodes (*P. redivivus* and *B. xylophilus*). Therefore, host preferences of nematode-trapping fungi

Table 5. Partial biochemical properties of serine proteases isolated from different nematode-trapping fungi.

Protease	Fungus	Mr[a] (kDa)	Optimal pH	Optimal TM[b]	Inhibitor	Reference
Mc1	*M. cystosporium*	38	7–9	56	PMSF	This study
Mlx	*M. microscaphoides*	39	9.0	65	PMSF	[22]
PII	*A. oligospora*	35	7–9	–	PMSF, pCMB	[21]
Aoz1	*A. oligospora*	38	6–8	45	PMSF, SSI	[28]
Ac1	*A. conoides*	35	7.0	53.2	PMSF	[25]
Ds1	*D. dactylella*	35	10.0	55	PMSF	[23]
Dv1	*D. varietas*	30	8.0	60.5	PMSF	[26]

[a]Molecular mass.
[b]Temperature.

(*e.g.*, *M. cystosporium*) for saprophytic and parasitic nematodes may help us explore nematophagous fungi as biocontrol agents against different nematode diseases.

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