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### Original Article

## Functionally Expression of Metalloproteinase in *Taenia solium* Metacestode and Its Evaluation for Serodiagnosis of Cysticercosis

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### **Abstract**

**Background:** Parasite proteases have important roles in cleavage of host proteins during the invasion of host tissues and participate in the parasite's evasion from the host's immune response. The aim of the present study was to estimate a metalloproteinase properties of *Taenia solium* metacestode (TsMP) during host-parasite interactions, and evaluate its potential as a serodiagnostic antigen for cysticercosis.

**Methods:** The cDNA coding for the mature catalytic domain of TsMP was cloned into pGEX-6P-1 expression vector. A recombinant glutathione S-transferase and TsMP fusion protein was induced. After refolding and purification, enzymatic properties of the recombinant metalloproteinase were observed. Immunoblot assay was processed to evaluate its potential as a serodiagnostic antigen for cysticercosis.

**Results:** The recombinant TsMP protein showed proteolytic activity, which preferred host extracellular matrix proteins such as collagen and fibronectin as degradable substrates. In immunoblot assay, 87.5% of sera from patients with cysticercosis showed strong reactivity. In sera from patients with other parasitic infections and from normal controls, it showed high specificity.

**Conclusions:** TsMP might be involved in the processing of numerous host proteins and play an important role in the parasite life cycle. A single recombinant TsMP antigen could have a potential value for serodiagnosis of cysticercosis.

## Introduction

Proteases are hydrolytic enzymes, which catalyse the cleavage of peptides and proteins. They are generally classified into four major classes: cysteine protease, serine protease, aspartic protease and metalloproteinase (1). These proteases play a number of critical roles in the parasite life cycle, involved in many aspects of host-parasite interactions. Proteases have an important role in cleavage of host proteins during the invasion of a host cell or host tissues and participate in the parasite's evasion from the host's immune response (2-4). Some parasite proteases have been successfully tested in experimental immunodiagnosis, proposed as major potential targets for immunotherapy and chemotherapy against parasitic diseases (5-8).

Many parasite metalloproteinases have been isolated and characterized until now. They are involved in a wide variety of adaptive functions as diverse as tissue penetration in *Ancylostoma caninum* (9-11) and *Onchocerca volvulus* (12), larval migration and larval molting in *Brugia pahangi* (13, 14) and *A. caninum* (10), degradation of cellular matrix in *Proteocephalus ambloplitis* (15, 16), immunomodulation in *Necator americanus* (17) and embryonic development and organ shape in *Caenorhabditis elegans* (18-20). Parasite metalloproteinase can also serve as immunodominant antigens, stimulating a protective immune response, or as potential targets for chemotherapy (21-23).

*Taenia solium* is the most common zoonotic parasite and cysticercosis caused by the infection of the parasite continues to be an important health problem worldwide, particularly in Central and South America, India, Africa, East Asia, Eastern Europe and other developing countries (24, 25). In the normal *T. solium* life cycle, infective eggs hatch, and the liberated oncospheres cross the membrane of the small intestine and migrate in the body, typically ending up in the central nervous system, skeletal muscle, subcutaneous tissue, or ocular

tissue. Within the host's tissues, the oncosphere matures into a cysticercus and causes the disease cysticercosis (26). Neurocysticercosis (NCC) is considered the most serious neurological disease in developing countries (27, 28). In previous work, we have isolated a membrane-associated metalloproteinase from *T. solium* metacestode (29). However, enzymatic function properties and the diagnostic capability of the protein, especially those of the recombinant protein, could not be properly evaluated. In this study, we expressed the mature catalytic domain of TsMP protein in prokaryotic expression system as GST-fusion proteinase. Enzymatic function of degradable host substrate of the purified recombinant proteinase were also observed to estimate its important role during the parasite development and the parasite-host interactions, and its potential as a serodiagnostic antigen for cysticercosis has also evaluated.

The aim of the present study was to estimate a metalloproteinase properties of *Taenia solium* metacestode TsMP during host-parasite interactions, and evaluate its potential as a serodiagnostic antigen for cysticercosis.

## Materials and Methods

### Parasite

*Taenia solium* metacestodes (TsM) were obtained from naturally infected pigs in an endemic area, Heilongjiang Province, China. Intact worms were washed over than five times with 0.87% cold physiological saline to remove any contamination from the hosts.

### Isolation of RNA and reverse transcription PCR (RT-PCR)

Fresh intact TsMs were ground in liquid nitrogen. Total RNAs were isolated by using Trizol reagents (Gibco, Carlsbad, CA). Poly (A)<sup>+</sup> RNAs were prepared from the total RNAs by oligo(dT)-affinity chromatography

(Qiagen, Valencia, CA). The first strand cDNA were synthesized from 1 µg of poly (A)<sup>+</sup> RNA by using a RNA PCR Kit (AMV) Ver. 3.0 (TaKaRa, Shiga, Japan) under the manufacturer's instruction. The specific primers were designed based on the *TsMP* gene encoding the putative mature catalytic domain of the TsMP protein (mTsMP) as recorded in GenBank (accession number DQ154010). The primer sequences were as follows: 5'-CCGTCGACCCAGATTGTGAATTGAGGAAGA-3' and 5'-TAGCGGCCGCTTACTTTTCCTTCCCATTTTAGC-3' (the underlined letters denoted the restriction enzyme sites of *Sal* I and *Not* I). The mature catalytic domain of *TsMP* was amplified by PCR with the specific primers using the cDNA as a template. PCR was carried out in a DNA thermal cycler (MJ Research PTC-100, Waltham MA) for 35 cycles with denaturation at 94°C for 50 sec, primer annealing at 58°C for 50 sec, and extension at 72°C for 2 min with a final extension 72°C for 10 min. The PCR products were analyzed by 1% agarose gel electrophoresis with ethidium bromide staining and purified with a QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) following the supplier's protocol.

#### Cloning and sequencing of mTsMP

The purified PCR product and pGEX-6P-1 vector (Amersham Biosciences) were digested with the corresponding restriction enzymes *Sal* I and *Not* I. They were ligated using T<sub>4</sub> DNA ligase (Promega, USA) overnight at 4°C, which was then transformed into the competent *Escherichia coli* DH5α cells by heat shock. The resulting white colonies were confirmed as containing the inserted sequence by colony PCR, restriction-enzyme analysis and further sequencing. We determined the nucleotide sequence by the dideoxynucleotide chain termination method, using the ABI Prism Big Dye Terminators v3.0 Cycle Sequencing Core Kit (Applied Biosystems, Foster City, CA) and an automated DNA sequencer (Applied Biosystems model 373 A; Perkin Elmer). The plasmid DNA of an inserted clone harboring

the expected coding sequence was extracted by QIAprep Spin Miniprep Kit (QIAGEN) and transformed it into *E. coli* strain BL21 (DE3) cells for expression.

#### Expression, purification and refolding of recombinant mTsMP

The transformed *E. coli* BL21 cells were grown in Luria-Bertani broth at 37°C to log phase, after which they were induced by adding 0.5 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG; Calbiochem) overnight at 30 °C. Solubility of the fusion protein was determined by suspending the *E. coli* in ice-cold PBS buffer (140 mM NaCl, 2.7 mM KCl, 10 mM Na<sub>2</sub>HPO<sub>4</sub> and 1.8 mM KH<sub>2</sub>PO<sub>4</sub>; pH 7.4). The bacterial-cells were lysed by sonication on ice (Sonics & Materials Inc. Vibra-Cell). The lysate was centrifuged at 15,000 rpm, 4 °C for 25 min. The supernatant was collected as soluble form and the pellet as insoluble form.

As the glutathione S transferase (GST)-tagged recombinant protein (GST- mTsMP) was expressed in its insoluble form; we attempted to solubilize the protein by dialyzing and refolding. The cell pellets were resuspended in 8 M urea solution containing 50 mM Tris-HCl (pH 7.5), 1 mM 1,4-dithiothreitol (DTT), and incubated 1 h at 4 °C. The solution was centrifuged and the supernatant was harvested into dialysis membrane (Spectro/Por® Membrane MWCO: 8,000) and then gradually dialyzed against 6 M, 4 M, 2 M and 1 M urea solutions at 4 °C. After that, dialysis was continued overnight against PBS buffer (pH 7.4) containing 5 mM CaCl<sub>2</sub>, 1 µM ZnCl<sub>2</sub>. Remove any visible insoluble material by centrifugation and the refolded recombinant mTsMP protein in supernatant was recovered. Refolded fusion proteins were then purified by Glutathione Sepharose 4B (GS4B, Pharmacia) chromatography and by ion-exchange chromatography with Q-Sepharose beads (Amersham Biosciences) according to the manufacturer's instructions. The purified recombinant mTsMP protein was then concentrated with Centricon Plus (cut-off 10 kDa; Millipore Billerica,

MA, USA), and confirmed by SDS-PAGE and western blotting with anti-GST antibody (Sigma) as primary antibody.

#### **SDS-PAGE and western blot analysis**

Twelve percent SDS-polyacrylamide gel was normally used to analyze the recombinant proteins. SDS-PAGE was performed in the Mini-Protean system (Bio-Rad). After electrophoresis, the gels were either Coomassie Brilliant Blue R250-stained to visualize the protein bands, or transferred onto a nitrocellulose (NC) membrane (Schleicher & Schuell BioScience, Dassel, Germany). The membrane was blocked for 1 h at room temperature with 5% (w/v) skim milk in TBS-T buffer (140 mM NaCl, 2.7 mM KCl and 25 mM Tris, pH 7.4; containing 0.05% (v/v) Tween-20). The membrane was incubated in mouse anti-GST antibody (Sigma) diluted 1:1,000 in TBS-T containing 5% (w/v) skim milk for 2 h at room temperature, and washed three times (each time for 10 min) in TBS-T buffer, followed by incubation with peroxidase-conjugated goat anti-mouse IgG antibody (Cappel, West Chester, PA) diluted 1:2,000 in 5% (w/v) skim milk. After washing, the ECL detection system (Amersham Biosciences) was added according to the manufacturer's instruction for 1 min. Treated NC membrane was exposed to an X-ray film.

#### **Zymograph (gelatin SDS-PAGE)**

Gelatin SDS-PAGE analysis was used to detect the recombinant mTsMP proteinase activity. In brief, 0.2% gelatin (w/v) was incorporated into 12% SDS-PAGE gels. Samples were run at 4°C under non-reducing condition and were not boiled prior to loading. After electrophoresis, gel was washed in 2.5% Triton X-100 for 1 h and reactivated for 18 h at 37 °C in 50 mM Tris-HCl (pH 7.5) buffer. Protein bands with proteinase activity in the gels were visualized by Coomassie Brilliant Blue R-250 using standard protocol. After destaining, the zone of proteolysis appeared as a clear white band in a blue gel. The effects of

inhibitors on proteinase activity was examined by using ethylenediamine tetraacetic acid (EDTA, 10 mM), phenylmethanesulfonyl fluoride (PMSF, 2 mM), *trans*-epoxysuccinyl-L-leucylamide (4-guanido) butane (E-64, 10 μM) and pepstatin A (1 μM). Before loading, the recombinant proteinase was preincubated with the respectively inhibitor for 30 min at 37 °C. The effects of metal ions (Ca<sup>2+</sup>, Zn<sup>2+</sup>, and Mg<sup>2+</sup>) in the incubation mixture on proteolytic activity were also determined by using the gelatin zymograph, no metal ion was used as control.

#### **Degradation of host proteins**

The recombinant mTsMP proteinase substrate specificity was detected by using several host substrates including purified collagen I (from human placenta), fibronectin (from human plasma), bovine serum albumin (BSA) and haemoglobin (from human serum). All substrates were purchased from Sigma (St. Louis, MO, USA). These substrate proteins (each 10 μg) were incubated with the purified recombinant mTsMP enzyme (1 μg) in 50 mM Tris-HCl (pH 7.5) buffer for 2 h or 4 h at 37°C. The reactions were terminated by adding the reducing sample buffer followed by boiling the mixture for 5 min. Digestion products were analyzed on a 10% SDS-PAGE.

#### **Preparation of anti-recombinant protein antibodies**

Five-week-old SPF BALB/c mice (female) were immunized subcutaneously (SC) with the purified recombinant mTsMP protein (30 μg per mouse) in an equal volume of complete Freund's adjuvant (CFA, Sigma). After two weeks, the mice were boosted immunization with recombinant mTsMP protein (30 μg per mouse) in an equal volume of incomplete Freund's adjuvant (IFA, Sigma) for two times at 2 weeks intervals. After 2 more weeks, the mice were finally boosted with the protein (10 μg in PBS). The mice were sacrificed 7 days later and blood was collected by heart puncture. The blood obtained from six mice was

pooled, allowed to clot and the antisera were collected. The immunoglobulin G (IgG) fraction was further isolated from the sera with a Protein G-Sepharose column (Amersham Biosciences, Piscataway, NJ, USA). The specificity of the antibody was confirmed by immunoblot analysis.

#### Determine the antigenicity of the recombinant mTsMP against patient sera

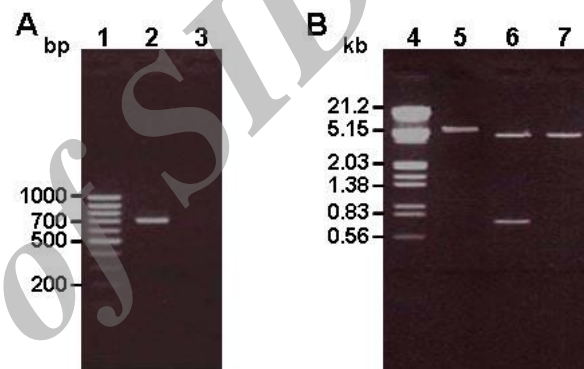
Immunoblotting was done using the serum samples from cysticercosis (n=40), echinococcosis (n=15), sparganosis (n=15), paragonimiasis (n=15), clonorchiasis (n=15) and the normal controls (n=15) to assess the diagnostic applicability of the recombinant mTsMP. The patients were diagnostic either by a stool examination (clonorchiasis), computed tomography of the chest and ELISA (sparganosis and paragonimiasis), or ELISA (cysticercosis and echinococcosis), combined with clinical symptoms compatible the respective disease. Control serum samples were obtained from healthy individuals. The recombinant protein was separated by 12% SDS-PAGE and was transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA). After being cut into strips, each strip was incubated overnight with the serum samples obtained from patients with different parasitic infections at a dilution of 1:200. Peroxidase-conjugated goat anti-human Immunoglobulin G (Cappel) was diluted at 1:1,000. The blots positive color reactions were visualized by incubating the membrane in PBS buffer (pH 7.2) containing 4-chloro-1-naphthol (4C1N, Sigma Chemicals) and 0.03% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>).

## Results

#### RT-PCR results and restriction enzyme analysis of the recombinant pGEX-mTsMP

Total RNA was isolated from fresh intact *Taenia solium* metacrestode. After reverse transcription, PCR of the putative mature catalytic

domain of TsMP resulted in an amplification fragment with a length of 717 bp (Fig. 1A). For the recombinant pGEX-mTsMP plasmid cut by *Sal* I and *Not* I, the results on 1% agarose gel revealed two bands of approximately 5 kb and 0.7 kb (Fig. 1B). The empty vector pGEX-6P-1 showed only one band demonstrating that the recombinant plasmid, pGEX-mTsMP, had an inserted fragment in it. DNA sequencing result showed that the inserted fragment completely matched the *TsMP* complementary DNA.

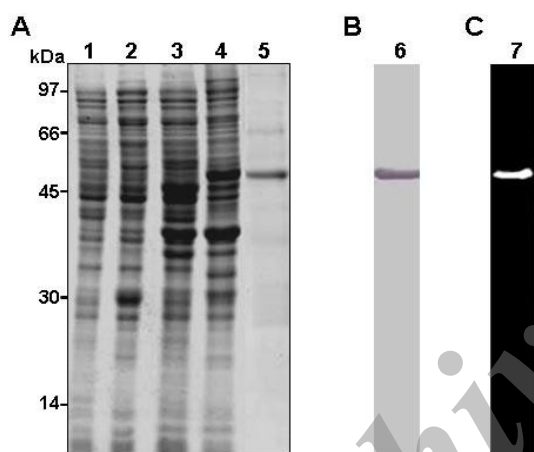


**Fig. 1:** RT-PCR amplification product for the mature catalytic domain of TsMP (mTsMP) cDNA (A) and restriction map of the pGEX-mTsMP recombinant plasmid by 1% agarose gel electrophoresis (B). Lane 1, DNA marker (100 bp DNA Ladder); lane 2, RT-PCR amplification fragment of mTsMP; lane 3, control group to confirm the absence of any contaminated DNA in the RNA sample by preparing reactions without reverse-transcriptase during synthesis of the first strand cDNA; lane 4,  $\lambda$ DNA /EcoRI + HindIII marker; lane 5, pGEX-mTsMP recombinant plasmid; lane 6, pGEX-mTsMP recombinant plasmid cut by *Sal* I and *Not* I; lane 7, pGEX-6P-1 empty plasmid cut by *Sal* I and *Not* I

#### Expression, purification and refolding of recombinant mTsMP protein

A mature catalytic domain of *TsMP* was amplified, cloned into the pGEX-6P-1 prokaryotic expression vector and transformed into *E. coli* BL21 (DE3). The recombinant mTsMP protein was expressed in *E. coli* as an insoluble protein with an apparent molecular mass of 52

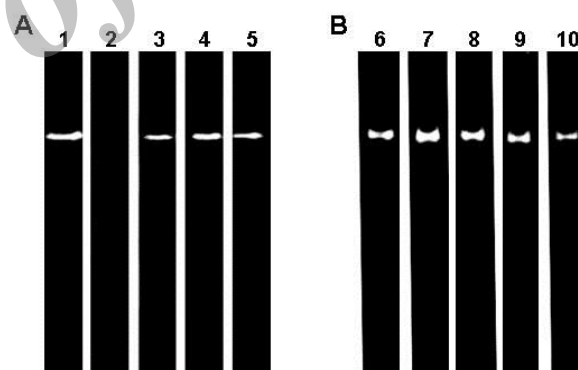
kDa (Fig. 2A), which is consistent with the estimated molecular mass of GST (26 kDa) and the deduced amino acid sequence for mTsMP (26 kDa). After dialysis and refolding, the recombinant protein was purified by Glutathione Sepharose 4B affinity chromatography and further purified by ion-exchange chromatography with Q-Sepharose beads. Western blotting result with anti-GST antibody as primary antibody demonstrated that the recombinant protein was the GST-fusion protein (Fig. 2B). Protease activity of the refolded recombinant GST-mTsMP was confirmed by substrate gel zymograph (Fig. 2C).



**Fig. 2:** SDS-PAGE, western blot and zymograph analysis of recombinant mTsMP enzyme. (A) Expressed protein were analyzed by 12% SDS-PAGE stained with Coomassie blue. Lane 1 and lane 3, soluble and insoluble fraction from *Escherichia coli* cell lysates which did not induce with 0.5 mM isopropyl-1-thio- $\beta$ -D-galactopyranoside (IPTG), respectively; lane 2 and lane 4, soluble and insoluble fraction from *E. coli* cell lysates which induced with 0.5 mM IPTG, respectively; lane 5, purified recombinant mTsMP protein by Glutathione Sepharose 4B affinity chromatography and ion-exchange chromatography with Q-Sepharose beads. The molecular weight marker in kilodaltons (kDa) was shown at the left. (B) Western blot analysis for purified recombinant mTsMP with anti-GST antibody as primary antibody (lane 6); (C) The activated recombinant TsMP was analyzed by substrate gel zymography with 0.2% gelatin as a substrate (lane 7)

### Effects of protease inhibitors and metal ions on enzymatic activities of recombinant mTsMP protein

Purified recombinant mTsMP protein was used to observe the proteinase properties. The effects of protease inhibitors on the recombinant proteinase were examined by gel zymograph. Zinc specific metal chelator EDTA (10 mM) strongly inhibited enzyme activity. It was uninhibited by serine proteinase inhibitor (PMSF, 2 mM), aspartic proteinase inhibitor (pepstatin A, 1  $\mu$ M) and cysteine proteinase inhibitor (E-64, 10  $\mu$ M) (Fig. 3 A). The addition of  $Mg^{2+}$  (2 mM),  $Ca^{2+}$  (2 mM) and  $Zn^{2+}$  (1  $\mu$ M) could enhance but did not significantly affect enzyme activity. However,  $Zn^{2+}$  in a concentration greater than 1 mM inhibited enzyme activity, with 5 mM  $Zn^{2+}$  resulting in an almost half reduction of proteinase activity (Fig. 3 B).

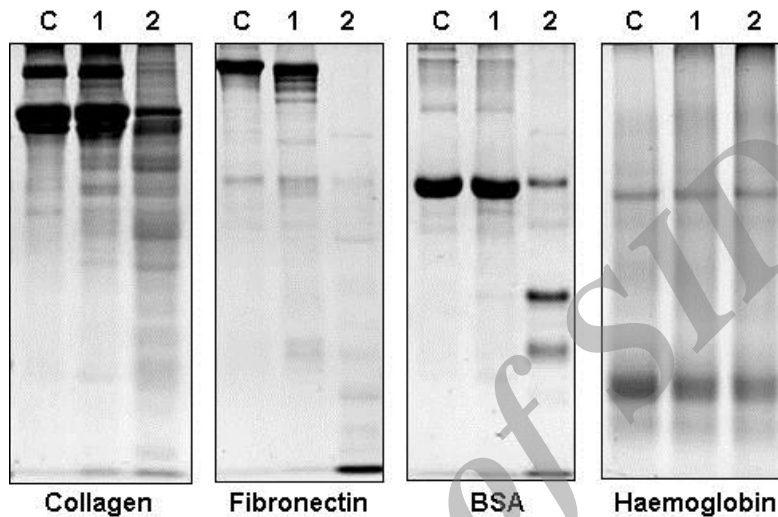


**Fig. 3:** Effects of protease inhibitor (A) and metal ion (B) on proteolytic activity of recombinant mTsMP protein by gel zymograph analysis. Lane 1, recombinant mTsMP; lane 2, recombinant mTsMP + 10 mM EDTA (metalloproteinase specific inhibitor); lane 3, recombinant mTsMP + 2  $\mu$ M E-64 (cysteine proteinase specific inhibitor); lane 4, recombinant mTsMP + 2 mM PMSF (serine proteinase specific inhibitor); lane 5, recombinant mTsMP + 1  $\mu$ M pepstatin A (aspartic proteinase specific inhibitor); lane 6, recombinant mTsMP; lane 7, recombinant mTsMP + 2 mM  $Ca^{2+}$ ; lane 8, recombinant mTsMP + 2 mM  $Mg^{2+}$ ; lane 9, recombinant mTsMP + 1  $\mu$ M  $Zn^{2+}$ ; lane 10, recombinant mTsMP + 5 mM  $Zn^{2+}$

### Degradation of host proteins of recombinant mTsMP protein

To investigate the putative biological roles of TsMP, the proteolytic activity of recombinant mTsMP against several host proteins including collagen, fibronectin, BSA and haemoglobin was determined.

All protein substrates except haemoglobin used in this study were hydrolysis by the recombinant mTsMP proteinase at neutral pH. However, the degradation of haemoglobin was not degraded significantly (Fig. 4).



**Fig. 4:** Degradation of various host proteins by the purified recombinant mTsMP enzyme. Each macromolecular protein substrate (10  $\mu$ g) including Collagen, Fibronectin, Bovine serum albumin (BSA) and Haemoglobin was incubated with recombinant mTsMP (1  $\mu$ g) in 50 mM Tris-HCl buffer (pH 7.5) for 2 h or 4 h at 37°C and analyzed by 10% SDS-PAGE. Lane C, control (protein without recombinant mTsMP); lane 1 and lane 2, incubated with recombinant mTsMP for 2 and 4 h respectively

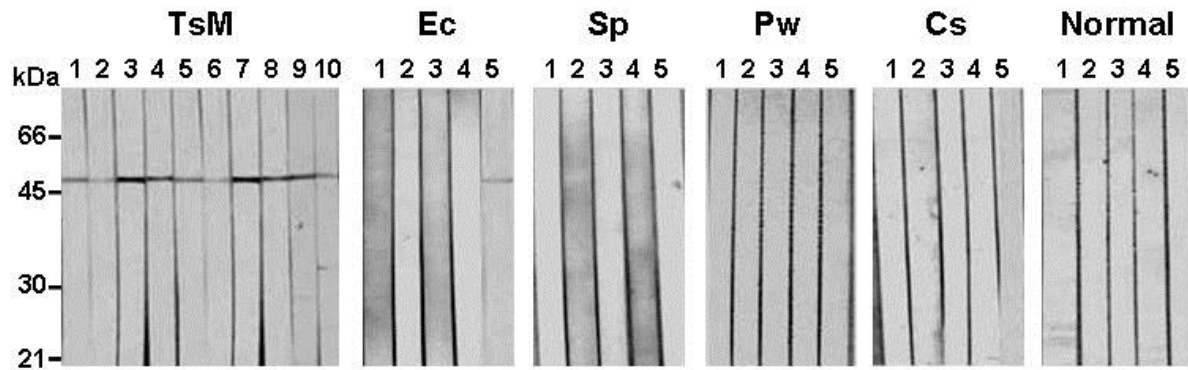
### Reactivity of recombinant mTsMP against serum samples from different parasite infections

To assess the diagnostic value of recombinant mTsMP, we tested its immunoreactivity by an immunoblot assay using individual sera from patients with various helminthic infections including cysticercosis, echinococcosis, sparganosis, paragonimiasis and clonorchiasis. A typical result of the immunoblot analysis is shown in Fig. 5. A strong recognition of recombinant mTsMP was observed in 87.5% (35/40 cases) of sera from patients with cysticercosis who were examined (Fig. 5, panel TsM). Meanwhile, weak cross-reactivity was observed in only 1 sample from a patient with echinococcosis (Fig. 5, panel Ec). None of the sera from patients with other parasitic infections

or from normal controls showed any antibody positive reactivity (Fig. 5, panel Sp, Pw, Cs and Normal).

### Discussion

In previous study, we have reported that TsMP, which isolated from *T. solium* metacestode, was a membrane-associated metalloproteinase structurally related to the FACE-1/Ste24p protease family, and the gene encoding the protein was identified (29). However, the putative enzymatic biological role and the diagnostic capability of the TsMP protein could not be properly evaluated. In the present study, we have examined its enzymatic partial function and evaluated its potential as a serodiagnostic antigen for cysticercosis.



**Fig. 5:** Immunoblot analysis of *Taenia solium* metacestode recombinant mTsMP protein against serum samples from patients with different parasitic infection. After transfer-blot of recombinant mTsMP separated by 12% SDS-PAGE, each strip was incubated with the respective serum in a dilution of 1:200, subsequently with 1:1,000 diluted peroxidase conjugated anti-human IgG. The colour reactions were developed with 4-chloro-1-naphthol (4C1N) and 0.03% hydrogen peroxide ( $H_2O_2$ ). *Panel TsM*, cysticercosis; *panel Ec*, echinococcosis; *panel Sp*, sparganosis; *panel Pw*, paragonimiasis; *panel Cs*, clonorchiasis; *panel Normal*, healthy controls. Lanes 1-10, each represent serum from different patient. The low molecular weight marker in kilodaltons (kDa) was shown at the left

To describe the potential function of native TsMP, the full length of TsMP was tried to express in a prokaryotic expression system by using *E. coli* and a yeast expression system by using *Pichia pastoris* (data were not shown). As they were both failed, the predicted mature catalytic domain of TsMP was further sub-cloned into prokaryotic expression vector (pGEX-6P-1). Although the recombinant mTsMP protein was expressed as an insoluble form, the soluble form of recombinant mTsMP was obtained by further refolding. After purified by using Glutathione Sepharose 4B affinity chromatography and Q-Sepharose ion-exchange chromatography, the refolded recombinant mTsMP protein was successfully obtained. Substrate gel zymograph assay with 0.2% gelatin as a substrate showed that the refolded recombinant mTsMP protein to be processed to an active enzyme (Fig. 2C). Many studies indicated that successful refolding of the expressed protein should be led to an active mini-enzyme with nearly the same properties as the wild-type enzyme (30-32). Therefore, properties of the recombinant mTsMP should be similar to the native TsMP. Proteinase inhibitor experiment

showed that recombinant mTsMP was most likely a metalloproteinase as it was strongly inhibited by the general metal chelator such as EDTA (Fig. 3A). The recombinant mTsMP enzyme was unaffected by other proteinase inhibitors such as the serine proteinase inhibitor, PMSF; aspartic proteinase inhibitor, pepstatin A and cysteine protease inhibitor, E-64 (Fig. 3 A). The relative enzymatic activity of recombinant mTsMP was slightly enhanced by the addition of 2 mM divalent metal ions ( $Ca^{2+}$  and  $Mg^{2+}$ ) (Fig. 3A), as has been observed for certain metalloproteinases (33, 34). Our results showed that zinc concentrations greater than 5 mM inhibited the some proteinase activity of recombinant mTsMP. Several zinc metalloproteinases are inhibited by excess zinc (35, 36). High zinc concentrations often inhibit metalloproteinase. The inhibition is due to formation of zinc monohydroxide that bridges the catalytic zinc ion to the side chain of the active site of the enzyme (35).

The substrate specificity experiment of the recombinant mTsMP protein indicated that it possesses proteolytic activity against natural host proteins such as fibronectin, gelatin, BSA, collagen. As shown in Fig. 4, fibronectin (a



non-collagenous glycoprotein component of the ECM), collagen (the major component of the basement membrane) and BSA (the most abundant serum protein in mammalian blood) were degraded by the recombinant mTsMP. These activities suggest that this proteinase may also play essential roles in the parasite biology, because all the assayed substrates are constitutive proteins of the hosts (pig, human) that *T. solium* metacystode parasitizes. Furthermore the TsMP enzyme can hydrolyzed some specific substrates such as collagen and fibronectin, which are also major components of gastrointestinal and muscle tissues, suggested this metalloproteinase could be play a major role in tissue degradation to facilitate parasite penetration or/and could be associated with larval migration process, as occurs with other parasite larvae (9, 37, 38).

The metalloproteinase protein could be available to diagnose the infected patients (21, 22, 39). In present study, we also noticed that the recombinant TsMP was highly sensitive and specific to react with sera of cysticercosis patients (Fig. 5). It indicated that a single recombinant TsMP antigen could have a potential value for serodiagnosis of cysticercosis, and TsMP may be an effective target molecule for vaccine development.

## Conclusion

TsMP might be involved in the processing of numerous host proteins and play an important role in the parasite life cycle. A single recombinant TsMP antigen could have a potential value for serodiagnosis of cysticercosis.

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