Parasitology Research (2025) 124:51
https://doi.org/10.1007/s00436-025-08499-9

RESEARCH

Construction, expression, and characterization of scFv fragment against *Fasciola gigantica* cathepsin L1H

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Received: 11 January 2025 / Accepted: 28 April 2025

Abstract

Fasciola spp. infection is a significant zoonotic disease. *Fasciola gigantica* cathepsin L1H (FgCathL1H) is expressed across the life stages of *Fasciola gigantica*: newly excysted juvenile (NEJ), juvenile, and adult. An emerging tool for diagnosing fasciolosis in humans and cattle involves single-chain variable fragments (scFv) antibodies. These antibodies, consisting of linked variable regions of heavy chains (VHs) and light chains (VLs), retain binding specificity and affinity. This study aims to construct, express, and characterize an scFv antibody for use in a diagnostic kit for fasciolosis. The study successfully constructed and expressed recombinant scFv antibody genes derived from mouse spleen cells in *Escherichia coli* HB2151. Specific VH and VL fragments targeting recombinant FgCathL1H were amplified, inserted into a phagemid vector (pCANTAB5E), and transformed into *E. coli* TG1. Infection with the M13KO7 helper phage produced recombinant phages, and scFv clones with a high binding capacity were selected through three rounds of bio-panning. The expression of scFv proteins was induced with 1 mM IPTG, yielding antibodies detectable in the culture supernatant and periplasmic space. The indirect ELISA revealed strong binding in 10 scFv phage clones, which were sequenced and analyzed via computer-guided homology modeling and showed a similar classification to CDR1–3, consisting of VHs and VLs. The scFv DNA construct was approximately 747 bp in length. The SDS-PAGE, ELISA, and western blot confirmed the specificity of the scFv clone 1B, particularly at ~ 29 kDa. Docking studies showed epitopes on the scFv interacting with FgCathL1H. This scFv reacted specifically with *F. gigantica* antigens at 36 kDa (whole body (WB) of metacercaria and NEJ) and ~ 28 kDa (WB of 4-week-old juveniles and adults, and adult excretory–secretory protein (ES)). Immunolocalization showed positive staining in the cecal epithelium. Thus, scFv anti-rFgCathL1H shows promise for diagnosing fasciolosis.

Keywords ScFv · *F. gigantica* · Cathepsin L1H · Recombinant antibody

Introduction

Fasciolosis, caused by trematode flatworms, is a parasitic infection that affects humans and a variety of mammals, especially animals involved in animal husbandry, such as cattle, sheep, and goats. *Fasciola hepatica* and *Fasciola gigantica* are endemic parasitic infections in several regions, such as Latin America, the Caribbean, Europe, the Middle East, Africa, Asia, and Oceania (Hotez et al. 2008). Fasciolosis occurs when humans or animals consume metacercaria (the infective stage) through contaminated vegetation or water, leading to the development of adult flukes in the liver (Mas-Coma et al. 2009). This disease can cause considerable economic losses in the livestock business, including milk and meat production (Spithill et al. 1999), while also posing a health risk to humans. Among the interesting

Section Editor: Robin Flynn

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Published online: 09 May 2025

cysteine proteases secreted by *F. gigantica*, cathepsin L1H (CathL1H) is the most potent antigen (Sansri et al. 2015). It exhibits significant functions at the interface between the parasite and the host. Furthermore, it is released when liver flukes defend themselves when living inside the host through the following processes: tissue invasion, the downregulation of type I response, the suppression of T cell proliferation, the degradation of the extracellular matrix (fibrillar collagen, types I and II) and the basement membrane (type IV collagen), the cleavage of immunoglobulins, larval excystation, and the digestion of host hemoglobin and liver tissue for nutritional purposes (Yang et al. 2023; Lecaille et al. 2002). Moreover, CathL1H is highly expressed in newly juvenile excysts (NEJs) and adult *Fasciola* spp. (Sansri et al. 2013). Therefore, it could be a perfect candidate antigen to be targeted by a recombinant antibody.

Monoclonal antibodies (MoAbs) have become essential components in the design and development of immunological diagnostic assays. The hybridoma approach has some limitations in the production of MoAbs, including reproducibility problems, the need for highly skilled animal handlers, and expensive production and quality control (Kohler and Milstein 1975). The stability of hybridoma cells and the time required for immunizing experimental animals are limitations in the production of MoAbs utilizing this technique. Moreover, hybridoma clones are at risk of losing their capacity to secrete antibodies (Frame and Hu 1990). The phage display technology is utilized in antibody engineering to help resolve the aforementioned issues and improve the reproducibility of the hybridoma technique. Therefore, the development of recombinant antibodies against rFgCathL1H is extremely important for diagnosis fasciolosis.

Recombinant monoclonal antibodies (rMoAbs) represent MoAbs that are generated by in vitro techniques using synthetic genes. The single-chain variable fragment (scFv) (~ 25–30 kDa) consists of variable heavy chains (VHs) and variable light chains (VLs), which are connected by a short peptide linker (Glockshuber et al. 1990; Ahmad et al. 2012). The scFv antibody is utilized in high-quality diagnostic tests. The recombinant antibody technology using phage display is a powerful tool to produce antibodies in large quantities (Ahmad et al. 2012). Moreover, this method has high specificity and low toxicity and does not need antibody-producing animals (Bazan et al. 2012). Recently, phage display has been developed in many formats such as Fab (Omar and Lim 2018), scFv, bispecific $F(ab)_2$ (Luthra et al. 2019), and scFv-Fc (Bujak et al. 2014). Furthermore, the rMoAbs have been developed and applied in several fields of research, such as medicine, biomedical science, immunology, cell biology, pharmacology, and drug discovery (Azzazy and Highsmith 2002), toxicological research in the pharmaceutical industry (Flisikowska et al. 2022), cancer therapies (Chester et al. 2004; Hudson 1999; Souriau and Hudson 2003), and immunodiagnostics (Raeisi et al. 2022). The gram-negative bacteria such as *E. coli* have already been successfully used to produce different antibody fragments (Raeisi et al. 2022). In order to construct the scFv antibody, mRNA is isolated from source cells such as hybridoma cells (Zhu et al. 2013) or spleen cells (Schaefer et al. 2010). The scFv antibody has been used to screen antibody fragments that recognize *F. hepatica* cathepsin B2 (CathB2) or antigens present in adult *F. hepatica* homogenates (Norbury et al. 2019a). Moreover, the scFv antibody against cystic echinococcosis was produced for the diagnosis of hydatid disease, a serious parasitic disease caused by the tapeworm *Echinococcus*. Another study showed that anti-HSP60 scFv from *Strongyloides* spp. has been used to detect parasitic antigens. The results showed increased diagnostic sensitivity and specificity (Miguel et al. 2020). A recent study produced an scFv antibody against *Opisthorchis viverrini* cathepsin F using phage technology for the diagnosis of opisthorchiasis (Martviset et al. 2024). Various studies have been conducted in multiple expression systems, such as mammalian cells and yeast cells (Ho et al. 2006), plant cells (Galeffi et al. 2006), and insect cells (Reavy et al. 2000).

This study aims to synthesize the antigen-binding site and develop mouse scFv antibodies, which are specific for rFgCathL1H. In this study, we cloned and developed a library of mouse scFv antibodies against rFgCathL1H antibody phagemid libraries that are bound to the rFgCathL1H. The scFv molecules were produced using engineering approaches to supplement or replace the antibodies produced by the hybridoma technique. In addition, scFv libraries are considered valuable due to their ability to avoid adverse immunological reactions. Moreover, their production cost is very low. Their advantages encompass reduced molecular weight and toxicity and enhanced thermostability, solubility, and diffusion properties. This study's results can be used to develop an antigen test kit in the future.

Material and method

Expression and purification of recombinant *F. gigantica* cathepsin L1H (rFgCathL1H)

The clone of rFgCathL1H (GenBank accession number: AY428949.1) with pET30b (+)/DE3 was kindly provided by the Research Unit for Vaccine and Diagnosis of Parasitic Diseases, Burapha University, Thailand. Briefly, the rFgCathL1H expression was induced by isopropyl-B-D-1-thiogalactoside (IPTG) (Calbiochem, Merck, Darmstadt, Germany) at a final concentration of 1 mM at 37 °C. For purification, the rFgCathL1H was purified using nickel–nitrilotriacetic acid (Ni–NTA) affinity chromatography (QIAGEN, Hilden, Germany) (Cheukamud et al. 2024).

The purified rFgCathl1H were collected and kept at − 20 °C for further experimentation.

Preparation mouse spleen and total RNA extraction

The experimental procedures adhered to ethical principles and guidelines for the utilization of animals and received approval from the Institutional Animal Care and Use Committee (IACUC) at Burapha University. Briefly, a 4-week-old female BALB/c mouse was immunized subcutaneously with rFgCathL1H/adjuvants at 2-week intervals. The rFgCathL1H protein was combined with the complete Freund's adjuvant (Sigma–Aldrich Inc., St. Louis, MO, USA) for the purpose of initiating the primary immunization process. The rFgCathL1H protein was combined with the incomplete Freund's adjuvant (Sigma–Aldrich Inc., St. Louis, MO, USA) at a concentration of 50 µg/100 µl for each immunization. Mice were euthanized 2 weeks after third immunization. The mouse's spleen was collected after euthanasia and cut to pieces approximately 0.5 × 0.5 cm in size. For total RNA extraction, Trizol reagent (ThermoFisher Scientific, Carlsbad, CA, USA) was immediately added to the spleen, and the tissue was homogenized and centrifuged at 12,000 g at 4 °C for 30 min. The supernatant was transferred to a new tube, and 200 µl of chloroform per 1 ml of Trizol reagent was added to the tube. The mixture was inverted for 2 min and then centrifuged at 12,000 g at 4 °C for 30 min. The aqueous phase was transferred to isopropanol, incubated for 10 min, and centrifuged at 12,000 g at 4 °C for 30 min. The RNA pellet was then resuspended in RNase-free water and stored at − 80 °C until further use.

Cloning of variable region genes

The total RNA was converted to complementary DNA (cDNA) using Revert Aid Reverse Transcriptase (Thermo Scientific, Lithuania). The cDNA of each VH and VL was synthesized using specific primers: MuIgG1/2 Reverse 5′ CTG GAC AGG GAT CCA GAG TTC CA 3′, MuIgG3 Reverse 5′ CTG GAC AGG GCT CCA TAG TTC CA 3′ (for VH), and MuCK Reverse Mix 5′ CTC ATT CCT GTT GAA GCT CTT GAC 3′ (for VL). The primers used in this study were designed based on primers from a previous study (Leger and Saldanhat 2000). The cDNAs encoding the VH and VL were amplified with polymerase chain reaction (PCR) using Q5 DNA polymerase (New England BioLabs Inc.). Sixteen primers were used for the amplification of the VH. The VH amplification (round I) consisted of 10 µl of 5X Q5 reaction buffer, 1 µl of 10 mM dNTPs, 2.5 µl of 10 µM forward primer (16 primers), 2.5 µl of 10 µM reverse primer (MUJHV, Rev mix), 1 µl of cDNA template, 0.5 µl of Q5 DNA polymerase, 10 µl of 5X Q5 high GC enhancer, and nuclease-free water (NFW) up to 50 µl, and these components were mixed. The MUVK forward primers (13 primers) were used for the amplification of the VL genes. The VL amplification (round I) consisted of 10 µl of 5X Q5 reaction buffer, 1 µl of 10 mM dNTPs, 2.5 µl of 10 µM forward primer (13 primers), 2.5 µl of 10 µM reverse primer, 1 µl of cDNA template, 0.5 µl of Q5 DNA polymerase, 10 µl of 5X Q5 high GC enhancer, and NFW up to 50 µl, and these components were mixed. The PCR products were analyzed and separated using electrophoresis in a 1% agarose gel with SYBR green and eluted using the QIAquick gel extraction kit (Thermo Scientific, Lithuania). The concentration and purity were determined using a NanoDrop spectrophotometer at wavelengths of A260 nm and A280 nm.

The VH and VL genes were subjected to a second round (set II) of PCR amplification. The primers for VH and VL that were applied in this amplification step were designed to include specific sequences belonging to the *Sfi*I restriction enzymes site for the VH gene and the *Not*I restriction enzymes site for the VL gene. The PCR products were analyzed and subsequently separated using electrophoresis in a 1% agarose gel with SYBR green. The resulting fragments were then purified using the QIAquick gel extraction kit (Thermo Scientific, Lithuania). The concentration and purity were assessed using a NanoDrop spectrophotometer at wavelengths of A260 nm and A280 nm.

Construction of scFv antibody anti-rFgCathL1H in expression vector

The concentrations of the VH-product and VL-product were determined, and they were subsequently pooled to a concentration of 10 ng/10 µl. The VH-product and VL-product were used as templates to assemble the scFv gene. A total of 10 µM forward primer scFv_*Sfi*I and 10 µM reverse primer scFv_*Not*I were used for the construction of scFv. The assembly product was separated in a 1% agarose gel and purified using the QIAquick gel extraction kit. The scFv gene was digested using *Sfi*I and *Not*I restriction enzymes (Thermo Scientific, Lithuania). The DNA was then ligated into a linearized phagemid vector (pCANTAB5E), which was digested with *Sfi*I and *Not*I restriction enzymes using T4 DNA ligase (Promega, USA). Digested products were examined using 1% agarose gel electrophoresis and subsequently extracted from an agarose gel using the QIAquick gel extraction kit. The pCANTAB5E-scFv phagemids were transferred in the *E. coli* XL1-Blue strain.

Rescue of phagemid library and bio-panning of phage antibody library

The pCANTAB5E-scFv phagemids were rescued by infection with an M13 KO7 helper phage. In brief, the transformed colonies were washed off with 2XYT broth. The

diluted culture was grown, and the M13 KO7 helper phage (New England BioLabs Inc.) was added at a MOI (phage/bacterial cell) of 20 and incubated at 37 °C and 250 rpm for 1 h. The culture was centrifuged at 4000 g and 25 °C for 20 min. The complete cell pellet was delicately resuspended in 2YT-ampicillin/kanamycin broth. After 14 h of incubation at 37 °C and 250 rpm, the culture was centrifuged at 10,000 g and 4 °C for 30 min. The supernatant containing the scFv phage library was collected and filtered through the 0.45-µm filter membrane. The 5X PEG/NaCl (Promega, USA) was added and incubated on ice for 1 h for precipitation. The precipitated phage pellet was collected using centrifugation at 10,000 g and 4 °C for 30 min. The pellet was dissolved using sterile 50% glycerol and kept at − 20 °C. The scFv phage library titer was determined for the bio-panning experiment.

The pCANTAB5E-scFv phagemid library was bio-panned for the selection of clones binding to rFgCathL1H. The immunosorbent tube (Maxi-sorb; Nunc, Roskilde, Denmark) was coated with 10 µg/ml rFgCathL1H, and another immunosorbent tube (without an antigen coating) was coated with 0.05 M coating buffer, and these tubes were incubated overnight at 4 °C. The immunosorbent tube was washed 3 times with 0.1% PBS-T (phosphate-buffered saline with 0.1% Tween 20) and blocked with 2% skim milk (PBS containing 2% skim milk) at RT for 2 h. To the immunosorbent tube without the rFgCathL1H antigen coating, the blocking (10^{11} scFv phage library, 1% skim milk, and 1% BSA diluted in 0.1% PBS-T) was added and incubated for 1 h. The blocking in the immunosorbent tube without an antigen was transferred to the immunosorbent tube coated with the antigen and incubated for 2 h. Then, the immunosorbent tube was washed 10 times with 0.1% PBS-T. After the unbound scFv phages were removed, the elution buffer (0.2 M glycine HCl) was added and incubated at 25 °C with shaking for 20 min. The pH was neutralized by the addition of 1 M Tris–HCl (pH 9.1). The scFv phages eluted into the solution were collected and transferred to a new microcentrifuge tube. The eluted scFv phages were filtered using a 0.2-µm filter.

One milliliter of the eluted scFv phage antibody was used to infect log-phase *E. coli* TG-1 at 37 °C and 250 rpm for 1 h and infected with *E. coli* HB2151 in the final panning round. The culture was freshly grown to a suitable optical density at 600 nm in 2XYT broth. The culture was centrifuged at 2000 g for 10 min. The *E. coli* cells were pelleted (2000 g for 10 min) and resuspended in 1 ml of 2XTY broth containing 100 µg of ampicillin (AMP) and 1% glucose (Glu) per ml. A 500 µl volume of scFv phage-infected *E. coli* was spread on a 2XYT/AMP agar plate and incubated at 30 °C for 16 h. The bacterial colonies growing on the 2XYT/ampicillin agar plate were counted to estimate the number of the eluted scFv phages. The colonies on the plate were rescued and used for further panning cycles. The 2nd and the 3rd panning were coated on immune tubes at concentrations of 1 µg/ml and 0.1 µg/ml, respectively; an increasing number of wash cycles were utilized 20 and 30 rounds, respectively. After the third round of bio-panning, 96 clones were randomly selected for positive clone analysis. To establish the number of eluted scFv antibodies, a 20 µl volume was taken from the suspension, and it was serially diluted on 2XTY agar plates containing 100 µg of ampicillin per ml. The dilutions were spread on 2XYT/AMP agar, and the bacteria were grown for 16 h at 30 °C.

Screening of scFv phage antibody binding clones by indirect ELISA

The rFgCathL1H was diluted in a coating buffer to a final concentration of 10 µg/ml (1 µg/well) and incubated at 4 °C overnight. Then, the 96-well plate (Thermo Scientific, Roskilde, Denmark) was washed 3 times with 0.05% PBS-T and blocked with 3% skim milk (SERVA Electrophoresis GmbH, Germany) in PBS and incubated for 2 h. Then, the 96-well plate was incubated with a culture supernatant of the scFv phage at 4 °C overnight. The 96-well plate was washed 3 times with 0.05% PBS-T. Then, rabbit anti-E-tag conjugated with horseradish peroxidase (HRP) (1:10,000) (ThermoFisher Scientific, Rockford, USA) was added and incubated for 1 h. Next, the 96-well plate was washed 3 times with 0.05% PBST. Then, 3,3′,5,5′-tetramethylbenzidine (TMB) (SeraCare Life Sciences, Milford, MA, USA) solution was added and developed for 15 min. The reaction was immediately stopped by adding 1 N HCl. The optical density (OD) of the reaction was measured at 450 nm using a SpectraMax® ABS microplate reader.

Anti-rFgCathL1H scFv antibody sequencing

The clones from the third round of panning were randomly picked from the 2XYT agar plate. Each colony was cultured in 2XYT broth and incubated overnight at 30 °C and 250 rpm. Plasmids were extracted from the bacterial pellets using the Miniprep kit (Qiagen, USA). The plasmids were analyzed by Macrogen (https://dna.macrogen.com/). The anti-rFgCathL1H scFv antibody gene cloned in the pCANTAB5E phagemid vector was sequenced using the pCANTAB5E-S1 primer (5′-CAACGTGAAAAAATTATTATTCGC-3′) and the pCANTAB5E-S6 primer (5′-GTAAATGAATTTTCTGTATGAGG-3′). The scFv-FgCathL1H sequences were analyzed and predicted for the complementarity-determining regions (CDRs) and immunoglobulin framework regions (FRs) using IMGT/V-QUEST (https://www.imgt.org/IMGT_vquest/analysis). Both the CDR-IMGT and FR-IMGT lengths of VH and VL sequences were based on the Collier-de-Perles tool. The sequence was translated using the Expasy translate tool (https://web.expasy.org/

translate/). Alphafold2 (https://colab.research.google.com/github/sokrypton/ColabFold/blob/main/AlphaFold2.ipynb) was used to predict the 3D structure. The Pymol program website was used to build the protein structure from the amino acid sequences in the 3D structure. The 3D model was docked using the free online server HADDOCK Web Server version 2.4 (https://wenmr.science.uu.nl/haddock2.4/). The PRODICGY webserver was utilized to predict the interactions of the scFv.

Expression of soluble anti-rFgCathL1H scFv antibody and periplasmic extraction

The soluble anti-rFgCathL1H scFv antibodies, an effective positive recombinant phage clone was chosen and used to infect log-phase *E. coli* HB2151 (OD_{600} = 0.5–0.6). The expression of soluble anti-rFgCathL1H scFv antibody was facilitated by adding 1 M isopropyl-B-D-1-thiogalactoside (IPTG) (Calbiochem, Merck, Darmstadt, Germany) to obtain a final concentration of 1 mM at 25 °C and 30 °C for 16 h. The culture was centrifuged at 2000 g and 4 °C for 20 min to collect the supernatant. The pellet was resuspended in 1XTES (0.2 M Tris hydrochloride, 0.5 mM ethylenediaminetetraacetic acid, and 0.5 M sucrose) and filtered through a 0.2-µm filter. Subsequently, 0.2XTES were added and mixed. The mixture was incubated on ice for 1 h, then centrifuged at 2000 g at 4 °C for 20 min. The supernatant containing soluble antibodies from the periplasm was collected and stored at − 20 °C.

Indirect ELISA and western blot analysis

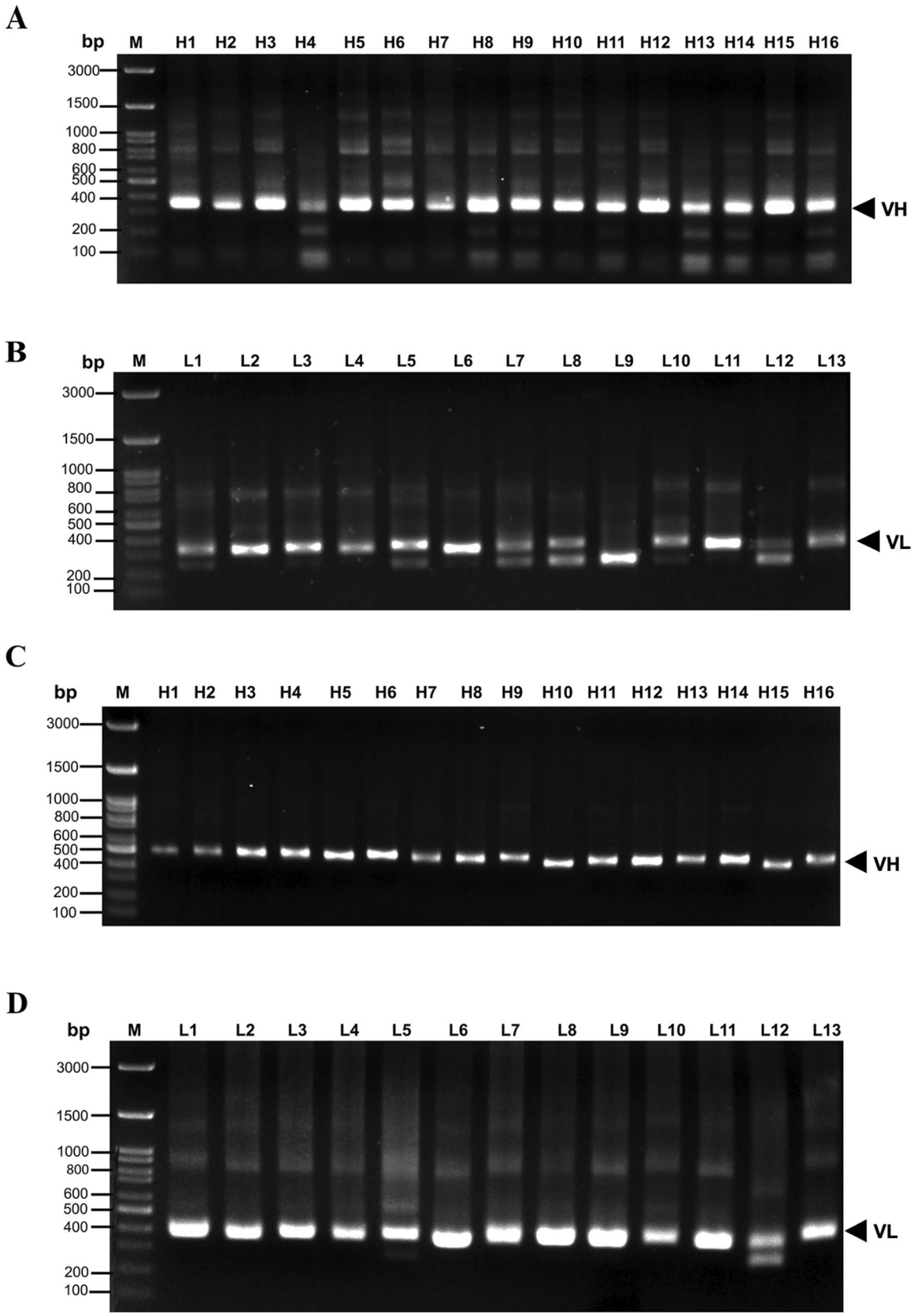
Whole body antigen (WB) extracts of *F. gigantica* at all stages (egg, metacercariae, newly excysted juveniles (NEJ), 4-week-old juveniles, and adults) and WB extracts of other species (*Eurytrema pancreaticum*, *Setaria labiatopapillosa*, *Paramphistoma cervi*, *Gastrothylax crumenifer*, *Cotylophoron cotylophorum*, and *Gigantocotyle explanatum*) were homogenized in a buffer containing 10 mM Tris–HCl, 150 mM NaCl, 0.5% Triton X-100, and 10 mM ethylene diamine tetra acetic acid (EDTA), pH 7.4, and sonicated for 5 min in an ice bath with 15-s pulses. Excretory–secretory antigens (ES) of adult *F. gigantica* were obtained by incubating adult parasites in Roswell Park Memorial Institute (RPMI) medium for 3 h at 37 °C. Tegumental antigens (TA) of adult *F. gigantica* were obtained by extracting the adult parasites with a non-ionic detergent (1% Triton X-100, 0.05 M Tris buffer, 0.01 M EDTA, 0.15 M NaCl, pH 8.0) at 37 °C for 20 min (Changklungmoa et al. 2014). After centrifugation at 10,000 g for 30 min at 4 °C, each antigen-containing supernatant was collected and stored at − 80 °C until used.

The parasite antigens (1 µl/ml) in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6) were coated and incubated in a 96-well plate at 4 °C overnight, as described in previous studies (Changklungmoa et al. 2018). Then, the plate was washed 3 times with 0.05% PBS-T and blocked with 3% skim milk. The scFv antibody was added to the 96-well plate and incubated at 4 °C overnight. Then, the plate was washed 3 times with 0.05% PBS-T, and the rabbit anti-E-tag conjugated HRP (ThermoFisher Scientific, Rockford, USA) (1:10,000) was applied and incubated for 2 h. Finally, 3,3′,5,5′-tetramethylbenzidine (TMB) (SeraCare Life Sciences, Milford, MA, USA) solution was added (50 µl/well) and incubated in a dark room at 25 °C for 15 min. Then, 1 N HCl was used to halt the reaction, and the plate was immediately read using a SpectraMax® ABS microplate reader at OD_{450}.

The antigen proteins (5 µg/well) were separated using a 12.5% SDS-PAGE gel and transferred to the nitrocellulose membrane. The nitrocellulose membrane was blocked with 5% skim milk for 2 h. After washing with 0.1% PBS-T, the anti-rFgCathL1H scFv antibody was applied to the membrane and kept at 4 °C overnight. The membrane was washed 3 times with 0.1% PBS-T for 15 min. The rabbit anti-E-tag conjugated with HRP (Thermo Fisher Scientific, Rockford, USA) was diluted to 1:2000, applied to the nitrocellulose membrane, and incubated at room temperature for 1 h. After washing, the enhanced chemiluminescence (ECL) substrate was applied to the nitrocellulose membrane and incubated for 1 min at room temperature and then developed. The nitrocellulose membrane was developed onto a film using the image software (Gel Doc™ EZ Imager, Biorad).

Immunolocalization

The adult *F. gigantica* section was dewaxed 3 times with fresh xylene for 5 min each and rehydrated. This section was rehydrated before staining using the following steps: absolute ethanol for 3 min, 95% ethanol for 3 min, 70% ethanol for 3 min, and tap water for 3 min. The tissue section was microwaved 3 times at 700 W in a citrate buffer (10 mM citric acid, pH 6.0) for 5 min. Next, the slide was directly washed with tap water for 5 min. The tissue section was incubated with 3% H_2O_2 in absolute ethanol for 30 min; then, it was washed 3 times with 0.1% PBS-T. A 4% BSA in PBS was applied on the slide and incubated for 1 h. The primary antibody (periplasmic extracted clone 1B) was diluted to 1:1 with PBS, added to the slide, and kept overnight at 4 °C. Next, the slide was washed 3 times with 0.1% PBS-T for 15 min to remove any unbound antibodies. The rabbit anti-E-tag conjugated HRP (1:200) (ThermoFisher Scientific, Rockford, USA) was added and incubated at room temperature for 2 h. Then, the slide was washed 3 times. Next, the section was incubated in the 3,3′-diaminobenzidine (DAB) substrate in a dark room. The reaction was stopped by adding distilled water. The


A
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VL

◂**Fig. 1** PCR amplification variable region genes (VH and VL genes) of mouse IgG amplified using primer set 1 (first round) was represented in **A** for VH and **B** for VL. The black head arrow represented the size of approximately 385 bp and 370 bp in both the heavy chain and the light chain, respectively. Round II of PCR amplification for VH and VL genes are shown in Figure **C** for VH and D for VL. The black head arrow represented the size approximately 400–500 bp and 370–500 bp in both heavy chain and the light chain, respectively. Lane M: GeneRuler^{TM}100 bp plus DNA ladder. Lanes H1–H16: amplification VH fragment from cDNA. Lanes L1–L13: amplification VL fragment from cDNA

slide was counterstained with Mayer's hematoxylin solution, mounted, and examined using light microscopy. The tissue section was photographed.

Results

Cloning variable region genes and scFv gene by PCR amplification

BALB/c mice were immunized with rFgCathL1H, and the total RNA of splenic cells was extracted. VH and VL were amplified using PCR. In the first round of amplification, all VH genes and VL genes were represented by separate PCR product bands of approximately 385 bp and 370 bp, respectively, on a 1% agarose gel, as shown in Fig. 1A, B, respectively. The products of VH and VL genes were amplified with primer set II to contain an adapter. Then, the VH and VL fragments containing *Sfi*I and *Not*I restriction enzyme sites were revealed on a 1% agarose gel, as shown in Fig. 1C (for VH) and D (for VL), respectively. After the amplification of the 2nd round using primer set II, the size of the VH was found to be approximately 400–500 bp. Furthermore, the size of the VL was found to be approximately 370–400 bp. The VH and VL gene products from set II were diluted to 10 ng, and the PCR products were pooled to separate the VH and VL products. Then, the pooled VH and VL genes were assembled to construct a single-chain variable fragment. The assembly of the scFv gene product using PCR overlap extension is shown in Fig. 2. The scFv antibody gene was appropriately 747 bp in length after its assembly using PCR amplification and its digestion using specific endonuclease *Not*I and *Sfi*I. The scFv anti-rFgCathL1H antibody was ligated into the phagemid vector named pCANTAB5E.

The phagemid containing scFv was transformed into the *E. coli* TG1 strain. The scFv antibody library was constructed by rescuing the transformed cells using the M13 KO7 phage. For the selection of the scFv antibody, three rounds of bio-panning were carefully performed.

Bio-panning and soluble scFv binding determining by indirect ELISA

The scFv antibody library was constructed by rescuing the transformed cells using the M13 KO7 phage. For the selection of the scFv antibody, three rounds of bio-panning were carefully performed. The supernatant collected from the clones of *E. coli* HB2151 expressing scFv was utilized for the determination of binding. The anti-rFgCathL1H scFv antibodies verified the expression of the scFv, and the positive clones were examined using the indirect ELISA, as represented in Fig. 3. Finally, the scFv clones contained an E-tag to enable their selection using the rabbit anti-E-tag conjugated HRP. The rFgCathLH was coated in an immuno tube and blocked with BSA to select the scFv phage with the antigen of interest. The first round of panning was coated with 10 µg/mL of rFgCathL1H. The second round and third rounds were coated with 1 µg/mL and 0.1 µg/mL, respectively. After the scFv phages were added and bound, the unbound phages were washed out. The bound phages were eluted and reinfected into *E. coli* TG1 (after the first and second rounds). However, in the third round, the scFv phages were reinfected into *E. coli* HB2151. After the third round of panning, the output titer slightly decreased from the first round to the third round (Table 1), which suggests that non-specifically binding phages existed in rounds 1 and 2. The positive phage clones were selected and eluted in three rounds. A total of 96 clones were obtained from the 3rd round of bio-panning, the library plate was selected randomly, and 86 clones were identified to be positive. However, a total of 10 positive phage clones exhibiting the highest OD_{450} values were selected from the chosen 86 phage clones for the purpose of sequence identification.

DNA sequence analysis and scFv-bound rFgCathL1H protein modeling

A positive clone was derived from the screening of cloned scFv phage library using the indirect ELISA, with rabbit anti-E-tag polyclonal antibody conjugated HRP for detection. The overnight culture was extracted for the plasmid DNA using the Miniprep kit (Qiagen, USA). The molecular interaction between the scFv antibody and the rFgCathL1H was studied in silico to better understand the properties of the scFv antibody fragment. All the phage clones shared the same amino acid sequence; thus, clone 1B was a group representative. Clone 1B exhibited significant differences, particularly in the CDR regions, indicating specific biological activities (Fig. 4A). The sequence was 747 base pairs long. Then, the amino acid sequences were translated and analyzed using the IMGT/V-QUEST server. The deduced amino acid sequence of the representative 249 amino acids consists of variable heavy-chain and variable light-chain

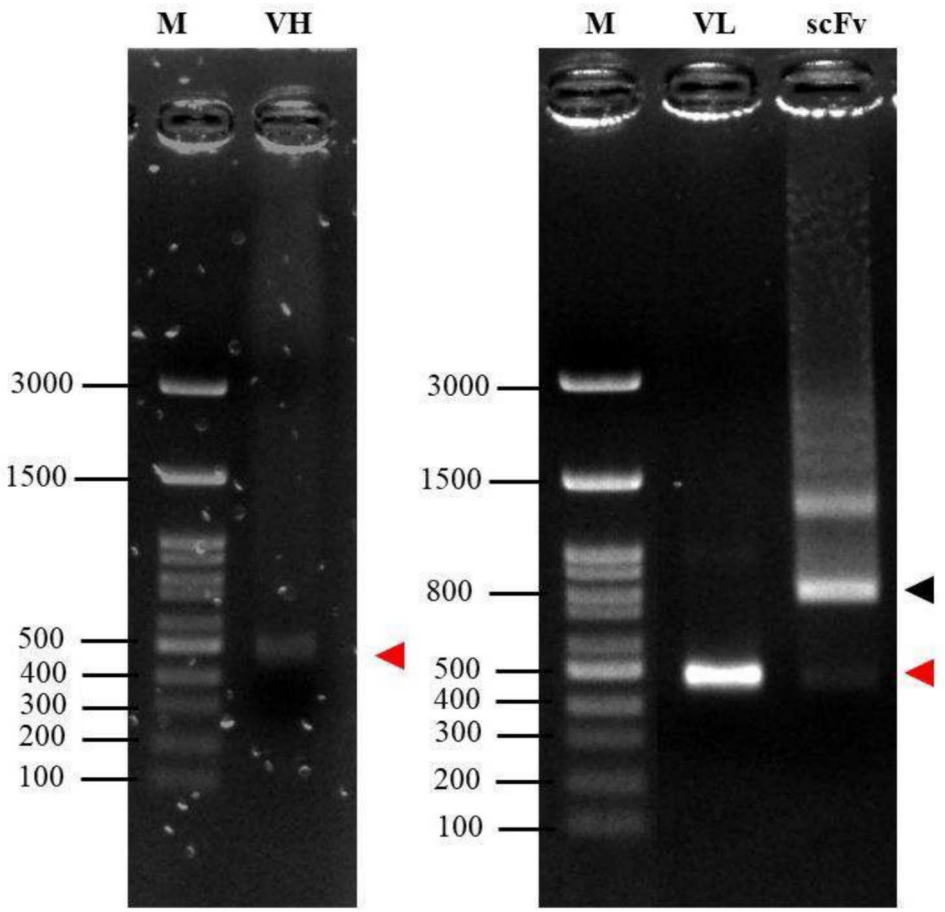


Fig. 2 Construction of scFv gene by a flexible glycine-serine (GS) linker. The pooled VH genes and VL genes were represented at approximately 500 bp (red arrow). The amplified PCR overlap extension product is separated on a 1% agarose gel and represented size approximately 747 bp (black arrow). M is GeneRuler™ 100 bp plus DNA ladder

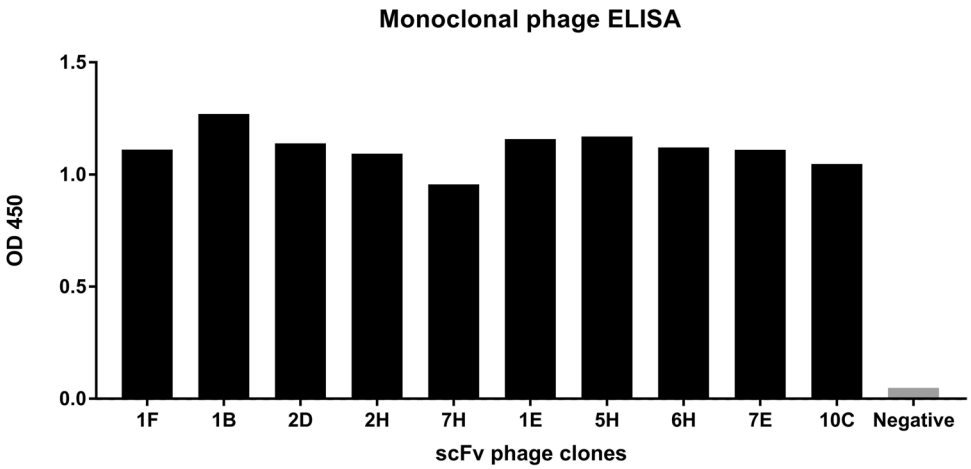


Fig. 3 Binding activity of the representative scFv phage clones. The bound anti-rFgCathL1H antibody was detected using rabbit polyclonal antibody anti-E tag conjugated HRP. Phage-ELISA different clones obtained from the 3rd round of panning. The culture media of different clones were added to each well of a microtiter plate pre-coated with rFgCathL1H A for characterized specific binding. The high OD_{450} of 10 clones were selected and shown

Table 1 The phage input and output titers over the 3 rounds of bio-panning of the mouse anti-rFgCathL1H scFv library

Panning	rFgCathL1H-coated (μg/ml)	Washes1XPBS-T (times)	Input phage (CFU)	Output (CFU/ml)
1 st round	10	10	1×10^{11}	1.93×10^{10}
2nd round	1	20	1×10^{11}	1.47×10^{10}
3rd round	0.1	30	1×10^{11}	1.02×10^{8}

translations of the scFv phage clone against rFgCathL1H, as shown in Fig. 4B and C, respectively. The predicted CDR 1, CDR 2, and CDR 3 of VH and VL domains were located at 27–38, 56–65, and 105–117 in the sequence. The amino acid sequence showed the complete scFv domain, containing the VH region, linker, and VL region, which is important mainly in the complementarity-determining (CDR) regions. The 3D structure of scFv anti-rFgCathL1H was generated by the Pymol program, as shown in Fig. 4D (the front side) and E (the back side). The 3D model of the molecular docking approach predicted the interaction between the scFv antibody and FgCathL1H, as shown in Fig. 4F, G. The predicted binding free energy (ΔG) and dissociation constant (K_d) were − 13.8 kcal mol^{-1} and $7.5e^{-11}$ M, respectively.

Soluble scFv against rFgCathL1H expression and analysis

Soluble single-chain variable fragments (scFvs) were generated through the expression of scFv phagemid within *E. coli* HB2151 cells. One out of ten clones that showed a strong signal (clone 1B showed the strongest signal) was selected for expression. In this study, we varied the induction temperature to optimize expression. The selected clone was cultured in 2XYT broth with shaking at 25 °C and 30 °C. The clone culture supernatant was harvested at 16 h post-induction using centrifugation at 4000 g and 4 °C for 10 min. After the expression of soluble scFv in *E. coli* HB2151, the non-induced culture, induced culture, culture media, and periplasmic extract were checked for the presence of scFv using a 12.5% SDS-PAGE gel, as shown in Fig. 5A. To investigate the expression of the scFv anti-rFgCathL1H, we transferred all the fractions to the NC membrane and analyzed them using western blot. The images in Fig. 5B summarize the specific scFv antibody expression. The results showed one expression band at approximately 29 kDa (indicated by a black arrow). In addition, the results showed that the lowering of culture temperature to 25 °C led to the improved soluble periplasmic expression of the scFv, as represented in Fig. 5B in lane 3 (the culture supernatant) and lane 4 (the periplasmic extract). However, at 30 °C, the autoinduction of recombinant antibody expression, as shown in Fig. 5B (lane 5), and a low expression in the periplasm, as shown in Fig. 5B (lane 8), occurred. It is believed that a strong gene expression can be induced in a low-temperature culture.

Characterization of scFv against rFgCathL1H

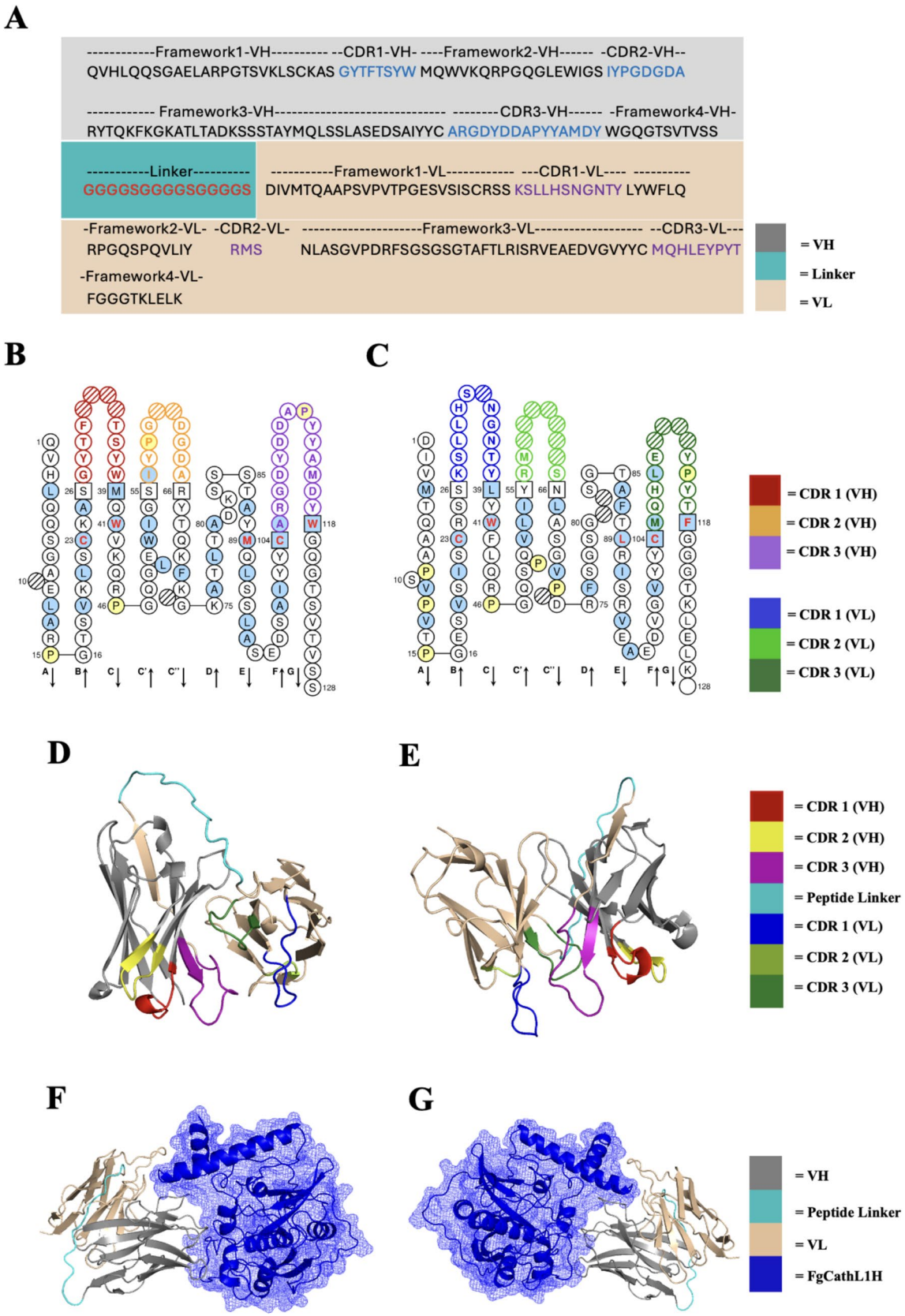
The reactivity of the periplasmic extract of the scFv antibody (clone 1B) was evaluated via indirect ELISA, western blot, and immunolocalization. The scFv secreted into the periplasm was analyzed, and it reacted with WB antigens from each stage of *F. gigantica* (egg, metacercaria, NEJ, 4-week-old juveniles, and adults) and the excretory–secretory (ES) antigen and the tegument antigen (TA) from adult *F. gigantica*. Significant increases in OD_{450} (cut off = 0.058) are observed in metacercaria, NEJ, 4-week-old juveniles, adult *F. gigantica*, and adult ES antigen when compared with the negative control (OD = 0.048), as represented in Fig. 6A. There was no significant reaction observed with the egg stage (OD = 0.048) and adult TA antigen (OD = 0.054). The results of the western blot are consistent with the results of the indirect ELISA. The metacercaria (meta) and newly excysted juvenile (NEJ) stages showed a specific band at approximately 38 kDa (a single black arrow in Fig. 6B). The WB of 4-week-old juveniles, adults, and adult ES antigen showed a positive band at approximately 25–28 kDa (a double black arrow in Fig. 6B). No positive band was detected in the egg stage and adult TA antigen.

Cross-reactivity was observed with other parasitic species, including bovine parasites such as *E. pancreaticum* (Ep), *S. labiatopapillosa* (Sl), *P. cervi* (Pc), *G. crumenifer* (Gc), *G. explanatum* (Ge), and *C. cotylophorum* (Cc). These parasites were collected from the infected organs, such as the rumen, bile ducts, and reticulum of cattle. The results showed that there was no cross-reaction with other parasites. The combined results of the ELISA (Fig. 6C) and western blot (Fig. 6D) support the conclusion that the scFv clone 1B is highly specific and suitable for diagnostic and therapeutic applications in fasciolosis.

The immunolocalization revealed the specific localization of the scFv antibody to adult *F. gigantica* antigens within tissue sections. Using the scFv clone 1B as the primary antibody, distinct and strong signals were observed in the cecal epithelium (Fig. 7B–D), indicating the presence of the targeted cathepsin L1H antigens. The scFv antibody was detected specifically in the cecum (Ca). In contrast, there is no signal detected from the tegument (Tg) and parenchyma (Pc).

Discussion

The phage display technique has been widely used to engineer the scFv antibody in various applications such as research, diagnostics, and therapeutic applications, and it offers several advantages over the hybridoma technology. In this technique, phages are constructed easily and cheaply by directly infecting *E. coli* (Wang et al. 1995), and these phages are more stable and can be stored at 4 °C for several years (Burritt et al. 1996). This study is the first to successfully clone and express the phage scFv library against FgCathL1H. The spleen of an immunized mouse was removed for the development of novel functional combinatorial antibodies that were expressed on the surface


A
----------Framework1-VH---------- --CDR1-VH- ----Framework2-VH------ -CDR2-VH--
QVHLQQSGAELARPGTSVKLSCKAS GYTFTSYW MQWVKQRPGQGLEWIGS IYPGDGDA
---------- Framework3-VH---------- --------CDR3-VH------ -Framework4-VH-
RYTQKFKGKATLTADKSSSTAYMQLSSLASEDSAIYYC ARGDYDDAPYYAMDY WGQGTSVTVSS
----------Linker----------
GGGGSGGGGSGGGGS
----------Framework1-VL---------- ---CDR1-VL---- ----------
DIVMTQAAPSVPVTPGESVSISCRSS KSLLHSNGNTY LYWFLQ
-Framework2-VL- -CDR2-VL- ----------Framework3-VL---------- --CDR3-VL---
RPGQSPQVLIY RMS NLASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYC MQHLEYPYT
-Framework4-VL-
FGGGTKLELK
= VH
= Linker
= VL
B
C
= CDR 1 (VH)
= CDR 2 (VH)
= CDR 3 (VH)
= CDR 1 (VL)
= CDR 2 (VL)
= CDR 3 (VL)
D
E
= CDR 1 (VH)
= CDR 2 (VH)
= CDR 3 (VH)
= Peptide Linker
= CDR 1 (VL)
= CDR 2 (VL)
= CDR 3 (VL)
F
G
= VH
= Peptide Linker
= VL
= FgCathL1H

◂**Fig. 4** Amino acid sequence of clone 1B (**A**).CDR-IMGT and FR-IMGT lengths are based on the IMGT/Collier-de-Perles tool results for variable heavy chain in **B** and variable light chain in **C.** The 3D structure of recombinant scFv antibody was visualized by Pymol program. The VH complementarity determining regions (CDR VH1-3) and VL complementarity determining regions (CDR-VL1-3) are labeled and shown in **D** (front side) and **E**(back side) using various colors to indicate their location. The structure of scFv consists of VH (gray stick) and VL (beige stick) joined together by peptide linker. Computerized docking interactions between modeled FgCathL1H and the scFv molecule in **F** (front side) and **G** (back side)by ClusPro 2.0

of bacteriophages. In previous studies, the scFv fragment of anti-*F. hepatica* cathepsin was generated from spleen cells (naive mouse) using phage techniques (Norbury et al. 2019b). The scFv fragment was ligated into the pCOMB3X phagemid vector and transformed into competent *E. coli* XL1-Blue cells. In other studies (Zhu et al. 2013), the scFv was produced from the hybridoma cell 6E6 that secretes the monoclonal antibody against the S protein of the porcine epidemic diarrhea virus. Moreover, the vector pGEX-6p-1 with a GST tag was used to generate rMoAbs and was expressed in *E. coli* using prokaryotic expression. When this study was compared with previous studies, it was evident that the phage display technique is a flexible method for creating antibodies against various targets, from viral proteins to parasitic antigens (such as *F. hepatica* and *F. gigantica*). This study also highlights the potential of the phage display technique to develop antibodies for diagnosing neglected tropical diseases, which have been often overlooked in antibody development. Several factors can affect the performance of the ScFv as a diagnostic antibody, including interference from host serum factors (e.g., serum proteins), its own stability (temperature, pH, storage conditions), and its binding affinity and specificity to the target antigen. Expression system variations, cross-reactivity with non-target molecules, and the optimal concentration of scFv also play a role in its diagnostic accuracy. Ensuring proper stability, minimizing interference, and optimizing conditions are key to enhancing its reliability.

Interestingly, we applied pCANTAB5E to express the scFv. However, many studies have used the spleen as a rich source of B cells that produce antibodies for various purposes, leveraging its abundance and diversity in antibody generation. The mouse IgG library was cloned using two primer sets, each consisting of 16 primers for the heavy chain and 13 primers for the light chain. The first set enables the amplification of individual B cell populations using mouse Ig genes. The second set is utilized to clone the amplified Ig gene into the expression vector using a second set of homologous primers that contain restriction endonuclease sites. In the cloning of the scFv gene, the VH and VL were with the linker and cloned into pCANTAB5E, located between the g3p leader sequence and the M13 gene 3. The M13 bacteriophage consists of coat proteins including g3p, g6p, g7p, g8p, and g9p. Thus, our scFv anti-rFgCathL1H antibody was present at the tip of the filamentous phage (Ledsgaard et al. 2018). Additionally, the g3p leader sequence directs the soluble scFv anti-rFgCathL1H antibody to the periplasmic space of *E. coli.*

Various studies have been conducted in multiple expression systems, such as mammalian cells and yeast cells (Ho et al. 2006), plant cells (Galeffi et al. 2006), and insect cells (Reavy et al. 2000). In recent years, a bacterial expression system has been developed to enhance the expression of recombinant proteins. Several studies have produced antibodies using *E. coli* HB2151 for the high-yield scFv production (Lalor et al. 2021; Ossysek et al. 2015) due to its oxidizing milieu that has facilitated the correct formation of disulfide bonds (Ghamghami et al. 2020). In this study, the scFv 1B antibody was efficiently produced by the *E. coli* strain HB2151 following IPTG stimulation and using the pelB-driven periplasmic transfer. The phage display technology has produced small peptides and antibodies fused with the pIII or pVIII coat proteins. Thus, we used rFgCathL1H as an antigen to screen and select the phage antibody library and for the bio-panning process. The binding affinity and specificity of the constructed scFvs were assessed through the ELISA. The phage ELISA result showed the enrichment of positive clones after increasing the duration of the washing step. Moreover, 2–3 rounds of panning lead to a higher number of high-affinity antibodies but reduced the diversity of these antibodies. The output colonies, which expressed the soluble scFv antibody with the E-tag, from each round of panning were screened and showed the expression of scFv antibody in the ELISA. An E-tag at the C-terminus enables immunodetection and purification using immunoaffinity (Xiong et al. 2009). The bio-panning method enables high-throughput selection, creates the binding sites for antibodies, and has good potential for use in many fields such as immunology, cell biology, pharmacology, and drug discovery (Azzazy and Highsmith 2002).

Nonetheless, this investigation has determined the ideal temperature for scFv antibody expression. Through the optimization of temperature, we decided to express the scFv antibody at 25 °C, as at 30 °C, low antibody levels were produced in the periplasm. In addition, the scFv antibody expression in a low-temperature culture at 25 °C significantly induced its high level of expression in the periplasm of *E. coli* HB2151. Autoinduction involves utilizing glucose and lactose as the primary carbon sources for cellular growth, with glucose being metabolized in the initial stages of *E. coli* growth (Blommel et al. 2007; Fox and Blommel 2009). Upon the near depletion of glucose, lactose becomes the preferred carbon source. This transition is coupled with a regioselective chemical conversion of lactose into allolactose. Allolactose, in turn, serves as the trigger for activating

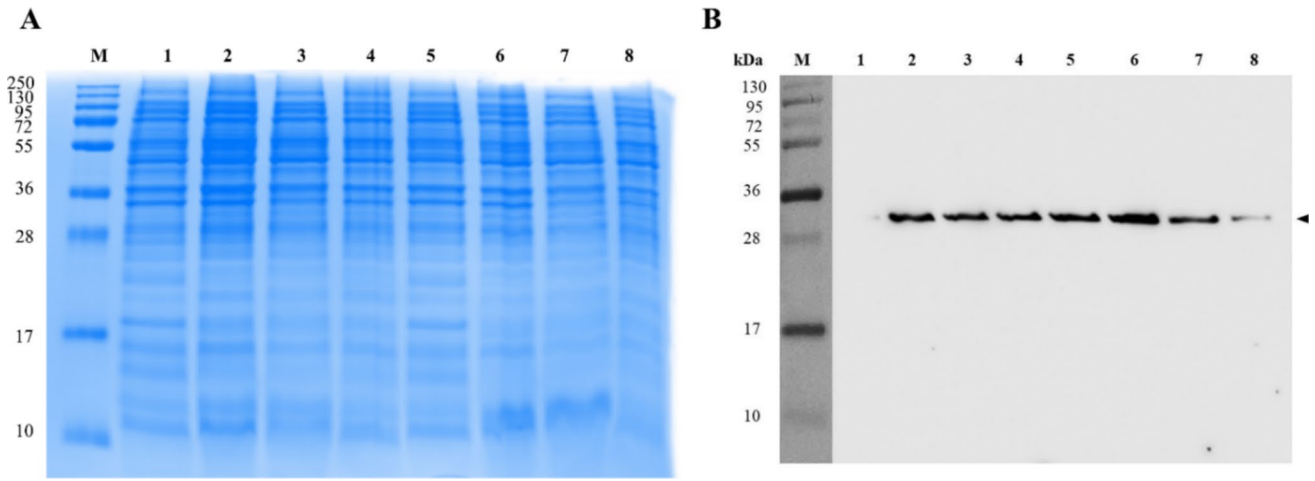


Fig. 5 Expression of recombinant scFv antibody anti-rFgCathL1H in *E. coli* HB2151 in different temperature. Coomassie blue staining to evaluate scFv expressed in *E. coli* HB2151 which were induced by 1 mM IPTG (**A**). Western blot analyses of scFv gene expression using rabbit anti E-tag conjugated HRP (**B**). Lane M shows molecular weight markers (kDa), lane 1 shows non-induce at 25 °C, lane 2 shows after induced with IPTG at 25 °C, lane 3 shows culture media at 25 °C, lane 4 shows periplasmic extract at 25 °C, lane 5 shows non-induce at 30 °C, lane 6 shows after induced with IPTG at 30 °C, lane 7 shows culture media at 30 °C, and lane 8 shows periplasmic extract at 30 °C, respectively. The scFv band is indicated with a black arrow. The anti-FgCathL1H scFv band represents 29 kDa. The position of individual scFv bands is indicated by a black arrow

transcription by releasing a repressor (Kataoka and Takasu 2021). Previous studies have used the TES extraction condition (50 mM Tris–HCl at pH 7.2 and 0.53 mM EDTA) and an incubation time of 60 min to improve the periplasmic scFv antibody production (Ghamghami et al. 2020). This protein can be found in the cell's reducing cytoplasmic compartment or in the oxidizing periplasmic compartment, which is located between the cytoplasmic and outer membranes. A strong promoter can produce high yields, and the presence of high protein content and the absence of disulfide bond formation (caused by a reducing environment) frequently result in the formation of insoluble inclusion bodies, which are characterized by misfolded protein precipitates. Usually, the *E. coli* strain HB2151 is successfully used to express the soluble scFvs, such as the scFv of murine McAb MC3 directed against colorectal and gastric carcinomas, because the amber stop codon in this strain worked as an interrupter between the g3p gene and the E-tag sequence, preventing the expression of fusion proteins (He et al. 2002).

Sequence analysis demonstrated that the VH and VL genes of the scFv were composed of four frameworks (FR1–FR4) and three complementarity-determining regions (CDR1–CDR3) that were highly specific to rFgCathL1H. A major contributor to antibody diversity is the highly variable sequence of the CDR3 region (D'Angelo et al. 2018). In this study, the VH amino acid sequence of CDR3, ARGDYDDAPYYAMDY, is deciphered, which shows 95.83% similarity with *Mus musculus* IGHV1-87*01 F1332 (accession number: NW_001033180); the translation amino acid sequence of CDR3-VL is MQHLEYPYT, which shows 99.32% similarity with the *Mus musculus* IgVk hf24 gene (accession number: AJ231263). Hosts require diverse antibodies to respond to the many different types of microbes or antigens. The variations in amino acid composition help receptor molecules in specifically binding to different antigens. The diversity of antibodies is combined with the V(D)J recombinant (variable, diverse, and joining regions) to form one V(D)J segment (Roth 2014). By experimenting with linkers of various lengths, we found that a $(Gly4Ser)_3$ linker provided an optimal balance between flexibility and rigidity, minimizing the risk of domain misfolding and aggregation (Turner et al. 1997). Somatic recombination is observed in the early stages of the light chain. Leader peptide (L), V, J, and constant (C) region exon are rearranged in the genomic DNA, but after the splicing of the light-chain RNA to remove L-to-V and J-to-C, the V-C region is joined and translated to produce the complete polypeptide light chain. The L sequence is removed after translation. On a heavy chain, the D and J genes are joined; then, the VH is completely formed by combining with the V gene. After the heavy-chain RNA transcript is processed, the C-region exons are spliced to obtain the V-domain sequence (Backhaus 2018; Schatz et al. 1992).

The reactivity of the soluble scFv 1B antibody was assessed using the anti-E-tag antibody with the indirect ELISA, western blot, and immunohistochemistry. The small volume of the *E. coli* periplasmic space limits the periplasmic yield of scFv, but the extracted protein from periplasm has many positive effects, such as facilitating the formation of disulfide bonds, having good folding properties,

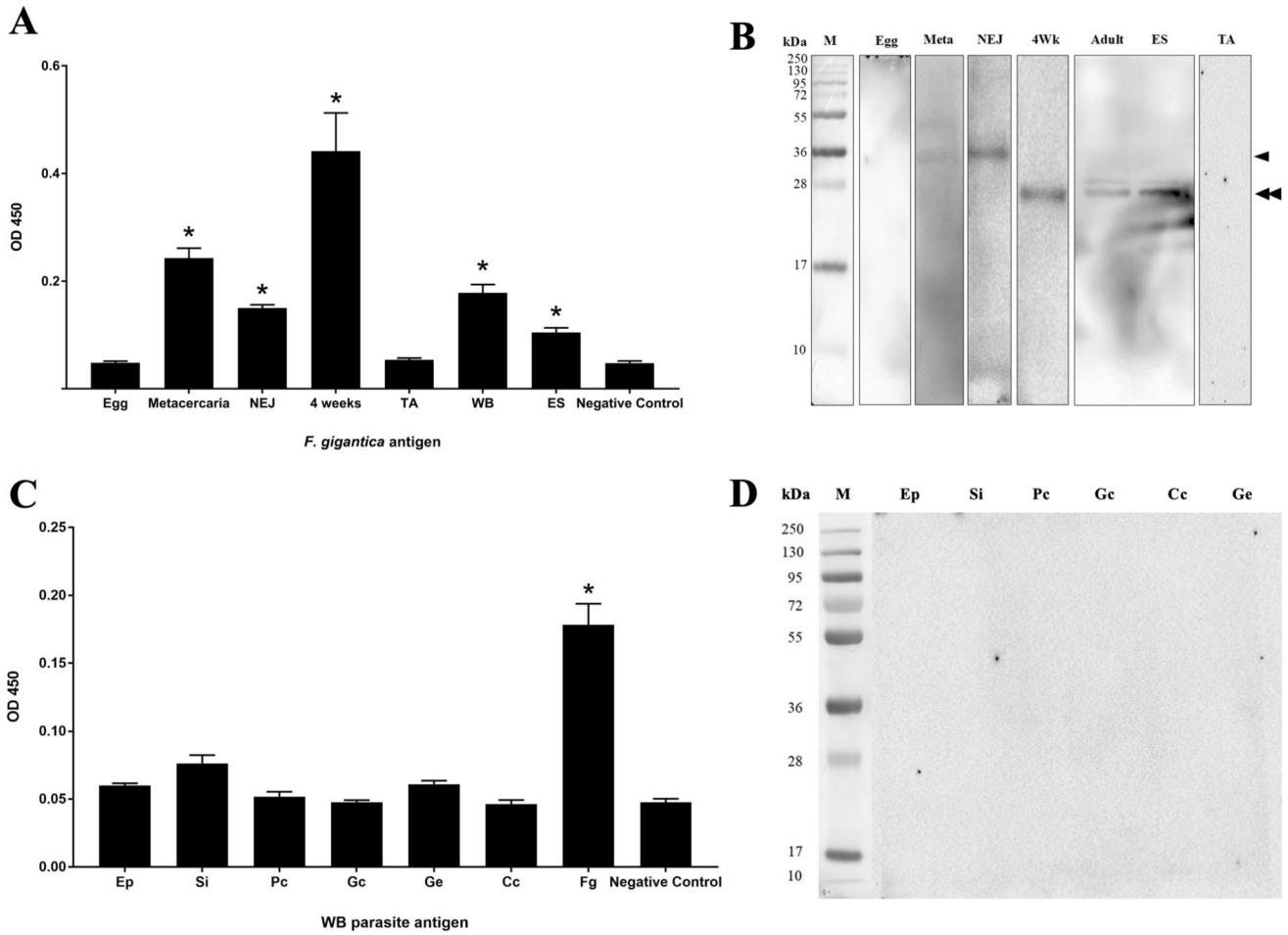


Fig. 6 Characterization of recombinant scFv antibody targeting with WB *F. gigantica* antigen (egg, metacercaria, NEJ, 4-week-old juveniles, and adults), ES and TA antigen of adult *F. gigantica* by ELISA assay (**A**). Western blot analysis of scFv antibody shows specific band of FgCathLs (**B**). Cross-reactivity analysis of scFv antibodies with antigens from other species by ELISA (**C**). Western blot studied for other species (**D**). No cross-reactivity to non-target species, confirming the antibody's specificity. *Significant increases were observed when compared with the negative control (p value < 0.05)

possessing low quantities of host proteins (reducing DNA contaminations), and preventing proteases from degrading intracellular proteins (Ghamghami et al. 2020; Gupta and Shukla 2016). In this study, the development and characterization of a scFv antibody is reported, which shows specificity for the rFgCathL1H, WB of NEJ, 4-week-old juveniles, adults, and adult ES antigen and has no cross-reactions with other species when tested in the indirect ELISA and western blot. In western blot analysis, the clone 1B scFv was confirmed using the rabbit anti-E-tag conjugated HRP, and a specific reaction band was found at ~28 kDa (in rFgCathL1H), which matched the theoretically predicted product. In IHC staining, the clone 1B scFv was confirmed and demonstrated the specific location of cathepsin L1s in the *F. gigantica* cecal epithelium. Our recent findings (Dalton et al. 2003; Wongwairot et al. 2015) corroborate our earlier findings, showing that the staining of scFv 1B is observed in the cecal epithelium. Thus, the scFv 1B antibody is a strong diagnostic candidate for fasciolosis. This study provides key data for future studies on the construction of scFv against FgCathL1H or the associated vaccine development.

Conclusion

In conclusion, this is the first study to accomplish the cloning and expression of antibody fragments targeting cathepsin L1H from the *F. gigantica* antigen. The successful implementation of the pCANTAB 5E vector enabled the efficient cloning and expression of a specific scFv antibody, anti-rFgCathL1H. The third round of bio-panning found 86 clones that show reasonable binding affinity towards rFgCathL1H. In this study, we found that 10 clones exhibited significant binding capabilities compared to the other clones.

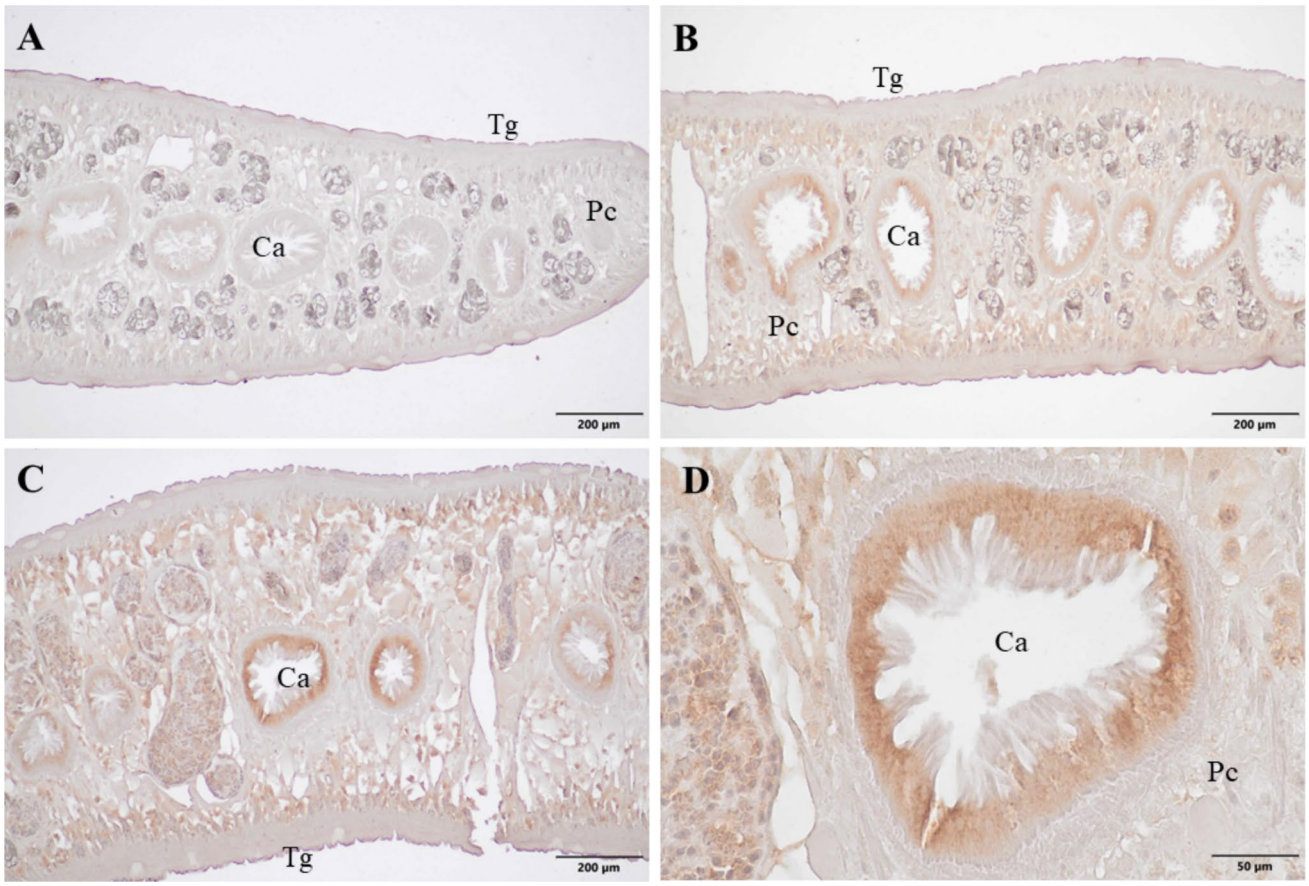


Fig. 7 Localization of adult tissues *F. gigantica* by immunohistochemistry detection using recombinant scFv antibody. The scFv antibody was specific and displayed strong at cecum of adult *F. gigantica*. **A** Negative control. **B**–**D** scFv antibody specified at cecum

After sequencing, all 10 clones showed identical deduced amino acid sequences. Therefore, clone 1B was selected as a sample for generating 3D structural models using the Pymol program website approach to highlight the complementarity-determining regions (CDRs) on the variable heavy-chain (VH) and variable light-chain (VL) fragments. Based on the experimental findings for the selection of antibodies with specificity to rFgCathL1H, the expressed antibody was found in the periplasmic space (the space between the outer and inner membranes of *E. coli*) and was subsequently secreted into the culture supernatant that was detected using the ELISA and western blot. No cross-reactivity was observed with other species. The scFv clones selected in this study have the potential to be engineered into alternative recombinant antibody formats and be used in the development of an immunodiagnostic kit in the future.

Supplementary information.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00436-025-08499-9.

Acknowledgements The authors would like to express my sincere gratitude to Research and Researchers for Industries (RRI) and Navi Science Co, Ltd. for their generous funding and support through the Grant no. PHD61I0045. This research would not have been possible without their financial assistance. I also would like to thank the Research Unit for Vaccine and Diagnosis of Parasitic Diseases, Burapha University for the support throughout this study. We are grateful to the Faculty of Allied Health Sciences, Burapha University for research facilities and supports.

Author contribution P.S. and N.C. wrote the main manuscript text, K.R., S.O., S.C. and A.P. performed the experiments, P.S. prepared figures 1-7, P.S., K.R., P.K., A.P., P.D. and N.C. analyzed the results, P.K. and N.C. edited the manuscript. All authors reviewed the manuscript.

Funding This research was funded by Research and Researchers for Industries (Grant no. PHD61I0045), co-funded by Navi Science Co, Ltd. and Research Unit for Vaccine and Diagnosis of Parasitic Diseases, Burapha University, Thailand.

Data availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval This study was approved by the Biosafety Board of Burapha University (IBC 020/2564) and Burapha University Institutional Animal Care and Use Committee (IACUC 001/2565).

Consent to participate Not applicable.

Competing interests The authors declare no competing interests.

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