

Recombinant single chain antibodies as an instrument to search proteins involved in the interaction of microsporidia and other intracellular parasites with an infected host cell

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Summary

Obligate parasitism of microsporidia - close to fungi protists causing widespread diseases of animals and immunodeficient patients, significantly complicates studies of their relationships with an infected host cell. Since microsporidia cannot be cultivated outside the host cell, genetic manipulations with them are extremely difficult. At the same time, long adaptation of microsporidia to the intracellular lifestyle, drastic minimization of their metabolic machinery, acquisition of unique transporters to exploit a host cell make these parasites a very valuable object for such study and require a search for new methods of investigation. Here, we describe our experiment on the construction of the library of recombinant single chain antibodies (scFv fragments) against proteins of fat bodies of locusts *Locusta migratoria* infected by the microsporidia *Paranosema (Antonospora) locustae*. The representativeness of this library was about 10^8 *E. coli* transformants carrying different combinations of variable fragments of heavy and light chains of immunized mice immunoglobulins. The first results of the selection of scFv fragments from the constructed library by phage display technology demonstrated that this approach may be effective to search proteins involved in the interaction of microsporidia and other intracellular parasites with an infected host cell. Cloning of selected genes into the expression vector, transformation of *E. coli* and screening two hundred bacterial colonies revealed scFv fragments against several such candidate proteins to begin their study. Further experiments with the library should discover new variants of recombinant antibodies interacting with the parasite and host proteins.

Key words: intracellular parasitism, host-parasite interactions, microsporidia, phage display, single chain antibodies, scFv fragments, *Paranosema locustae*, *Locusta migratoria*

Introduction

Microsporidia are closely related to fungi obligate intracellular parasites that cause widespread diseases of insects, crustaceans, mussels, fishes, homiotherm animals and humans with weakened immunity (Didier, Weiss, 2008). Despite the interest of scientists to microsporidia and various microsporidiosis, the molecular aspects of their relationships with an infected host cell remained poorly studied for a long time. This is primarily due to inability to cultivate microsporidia outside the host cell and to the difficulty of carrying out any genetic manipulations with these microorganisms. At the same time, this group of parasitic protists is one of the most promising objects for such research. Sequencing of microsporidian genomes has shown a unique minimization of their metabolic machinery (Katinka et al., 2001; Keeling et al., 2010) and acquisition of unique transporters to effectively exploit an infected host cell (Tsaousis et al., 2008; Heinz et al., 2014; Dean et al., 2018). Finally, most species of microsporidia develop in direct contact with the cytoplasm of an infected cell and like the piroplasms *Theileria annulata* (Swan et al., 2001; Shiels et al., 2004) should control the hosts molecular and biochemical processes.

Several approaches were used to study proteins involved in relations of microsporidia with infected host cells. Sequencing of their genomes revealed a variety of proteins with N-terminal signal peptide responsible for secretion. Heterologous expression and production of polyclonal antibodies against recombinant products make possible to get valuable data on their localization and functions (Dang et al., 2013; Senderskiy et al., 2014). However, such study of individual proteins is not a comprehensive one. Besides, it does not allow the analysis of expression of host proteins in response to infection. Expression of multiple genes in the infected by microsporidia and control tissues of silkworms (Ma et al., 2013) and honey bees (Holt et al., 2013) was compared using the methods of transcriptomics. Another approach based on the methods of proteomics was used to analyze the proteins that are released by the microsporidia *Spraguea lophii* during spore germination and can be involved in host-parasite relationships (Campbell et al., 2013). Unfortunately, neither transcriptomic nor proteomic methods provide any information on the localization of studied proteins in parasite or host cells. The peculiar

complex method to search proteins interacting with an infected cell was developed for microsporidia of the genus *Nematocida* (Reinke et al., 2017). The authors expressed ascorbate peroxidase in the cytoplasmic or nuclear compartments of the nematode *Caenorhabditis elegans*. Isolation and mass spectrometric analysis of biomolecules biotinylated by the enzyme in the presence of biotin-phenol and hydrogen peroxide identified 82 parasitic proteins contacting with an infected host cell.

Another approach for the complex analysis of microsporidia secretomes may be based on the use of recombinant single-chain antibodies (scFv fragments). Single-chain variable fragments (scFv) are 25-27 kDa fusion proteins consisting of the variable regions of the heavy (VH) and light (VL) chains of immunoglobulins, connected with a short flexible linker peptide of 10 to about 25 amino acids. This protein retains the specificity of the original immunoglobulin, despite the removal of the constant regions and the introduction of the linker. After cloning of pools of VH and VL genes in special phagemid vectors, specific scFv-variants may be selected from constructed libraries by phage display technology and effectively produced in bacterial cells (Sheets et al., 1998; Okamoto et al., 2004). Since scFv fragments may be genetically fused to recombinant tags (Ikonomova et al., 2016), their immobilization on commercial resins in the correct spatial orientation allows to isolate new proteins for further analysis and identification.

To assess the efficiency of scFv-fragments for searching of biomolecules involved in the relationships between microsporidia and infected host cells we constructed two libraries of scFv fragments against proteins of fat bodies of the locusts *Locusta migratoria* infected with the microsporidia *Paranosema (Antonosporea) locustae*. Representativeness of the first library was relatively low – about 5×10^5 *E. coli* transformants carrying different combinations of variable fragments of heavy (VH) and light (VL) chains of immunized mice immunoglobulins. Nevertheless, we have selected from this library scFv-fragment recognizing a/b-hydrolase – one of the most interesting *P. locustae* protein secreted into infected host cells (Dolgikh et al., 2017). Here we reported about the construction of the more representative library of scFv-antibodies against the proteins of infected locust fat bodies and selection of new variants of scFv mini-antibodies.

Material and methods

To amplify sequences encoding VH and VL fragments of immunoglobulins we used frozen at -80°C spleen of mice immunized for the creation of the first library (Dolgikh et al., 2017). The construction of the second library, selection of the phages, expression of scFv antibodies in *E. coli* and analysis of their antigen-binding activity were performed as described in the previous study (Dolgikh et al., 2017). To create a much more representative library, we scaled up about 200 times the amount of isolated total RNA, synthesized cDNA, amplified VH/VL genes, and prepared plasmid DNA. Because of the huge number of transformants we grew them in a liquid medium instead of Petri dishes.

CONSTRUCTION OF A LIBRARY OF RECOMBINANT ANTIBODIES

Total RNA of the spleen of the immunized animals was isolated using Trizol reagent (Thermo Fisher Scientific, MA). Synthesis of cDNA was carried out for 1 h at 37°C in PCR tubes with $20\ \mu\text{L}$ of the reaction mixture containing $2.5\ \mu\text{g}$ RNA, $10\ \text{mM}$ Tris-Cl (pH 8.8), $50\ \text{mM}$ KCl, $5\ \text{mM}$ MgCl_2 , $1\ \text{mM}$ of each dNTP, $1\ \mu\text{g}$ oligo (dT) as a primer, $200\ \text{U}$ of RevertAid M-MuLV-reverse transcriptase (Thermo Fisher Scientific, MA) and $5\ \text{U}$ of RNAase inhibitor (Thermo Fisher Scientific, MA). Then, the mixture was heated at 95°C for 5 min, and $1.6\ \mu\text{L}$ of it was used for PCR performance. In addition to cDNA, the PCR mixture ($20\ \mu\text{L}$) contained $67\ \text{mM}$ Tris-Cl (pH 8.6), $2.5\ \text{mM}$ MgCl_2 , $16.6\ \text{mM}$ $(\text{NH}_4)_2\text{SO}_4$, $0.5\ \text{mM}$ of each dNTP, $10\ \text{pmol}$ of primers, and $2.5\ \text{U}$ of Taq DNA polymerase (Sileks, Russia). For amplification of all diverse sequences of VH and VL genes of mouse IgG chains, 22 combinations of the specially chosen primers (Progen Biotechnik, Germany) were used. The matrix was denatured for 3 min at 94°C , and DNA was amplified over 30 cycles, each of which included denaturation (30 s at 94°C), annealing (30 s at 55°C), and synthesis (30 s at 72°C). PCR products of approximately 400 bp were isolated from agarose gel and used as a matrix for the second PCR round with primers containing restrictase sites on the 5'-terminus for incorporation into the vector pSEX81 (Progen Biotechnik, Germany).

PCR products encoding VH fragments were isolated from agarose gel after electrophoresis,

pooled together, and inserted into the vector by restrictase sites NcoI and HindIII. *E. coli* cells (XL1-Blue MRF' strain) were transformed with the obtained constructs through electroporation.

A small aliquot of bacterial suspension after each electroporation (1/100 volume) was sown on plates with selective media to evaluate the resulting number of transformants. The most part of transformed bacteria was grown in $2\times\text{YT}$ liquid medium containing $100\ \mu\text{g}/\text{mL}$ ampicillin and $0.1\ \text{M}$ glucose ($2\times\text{YT-GA}$), for 24 h at 30°C , harvested by centrifugation and stored in $2\times\text{YT-GA}$ with 25% glycerol at -80°C . Repeating the transformation procedures many times allowed us to obtain sub-library consisting of about 10^8 transformants. To insert VL genes into the sublibrary, plasmid DNA was isolated by alkaline lysis, linearized by MluI/NotI restrictases, and ligated with PCR-amplified VL fragments treated with the same enzymes. Repeating bacteria transformation by the ligation mixture for many times allowed us to obtain the final library consisting of about 10^8 transformants. Bacterial cells of this library were cultivated in $2\times\text{YT-GA}$ at 30°C for 24, harvested by centrifugation and stored in the same medium with 25% glycerol as $200\ \text{mL}$ of suspension with OD_{600} (optical density at wavelength 600 nm) about 6 units.

SELECTION OF RECOMBINANT scFV-FRAGMENTS

To produce phage particles, a part of the library was diluted in $200\ \text{mL}$ of $2\times\text{YT-GA}$ to $\text{OD}_{600}\ 0.025$, grown up to $\text{OD}_{600}\ 0.1$ at 37°C and infected with a helper Hyperphage M13 K07 Δ pIII (Progen Biotechnik, Germany) at a rate of about 200 phages per bacterial cell. After incubation at 37°C for 20 min without stirring and 50 min with stirring at an orbital shaker (260 rpm), the medium was replaced by that $2\times\text{YT}$ with $100\ \mu\text{g}/\text{mL}$ ampicillin and $50\ \mu\text{g}/\text{mL}$ kanamycin and the bacterial culture was incubated at 260 rpm overnight at 37°C . After precipitation of bacteria by centrifugation the supernatant was mixed with a one-fifth volume of 20% PEG 6000 containing $2.5\ \text{M}$ NaCl. After 1 h incubation of the suspension on ice, phage particles were precipitated by centrifugation at $14000\ \text{g}$ for 20 min at 4°C , resuspended in $3\ \text{mL}$ of phage dilution buffer ($10\ \text{mM}$ Tris-Cl (pH 7.5), $20\ \text{mM}$ NaCl, and $2\ \text{mM}$ EDTA) and stored at -80°C .

To select the phages with specific antibodies, the proteins of fat bodies of infected locusts were immobilized on CNBr-activated Sepharose 4B

(GE Healthcare Life Sciences, MA) instead of MaxiSorp polystyrene tubes (Nunc, Denmark) used in previous experiments (Dolgikh et al., 2017). To prepare antigen, fat bodies of infected locusts were gently disrupted in phosphate-buffered saline (PBS; 138 mM NaCl, 3 mM KCl, 1.5 mM KH_2PO_4 , 8 mM Na_2HPO_4 , pH 6.8) using a glass homogenizer and a freely matched Teflon pestle. Spores and intracellular stages of microsporidia were pelleted at 100 g for 10 min, the supernatant was further centrifuged at 20000 g for 20 min and the soluble fraction was carefully dialyzed against the binding buffer (0.1 M Na-carbonate buffer (pH 9.0), 0.5 M NaCl). Approximately 0.3 mg of dialyzed protein was incubated for 2 h at room temperature with 0.45 mL of CNBr-activated Sepharose swollen in 1 mM HCl. The reaction was carried out in 1.5 mL microcentrifuge tubes on a mini-rotator and resin was washed with binding buffer. Active sites were blocked for 2 hours in the presence of 0.1 M Tris-Cl (pH 8.0) and Sepharose was washed with 5 volumes of 0.1 M Na-acetate (pH 5.2), 0.5 M NaCl and then with 5 volumes of 0.1 M Tris-Cl (pH 8.0), 0.5 M NaCl. This procedure was repeated three times, Sepharose was resuspended in Tris-buffered saline (TBS: 50 mM Tris-HCl (pH 7.5), 150 mM NaCl), aliquoted in three tubes and used for three rounds of selection.

To start the first round of panning thawed phage suspension was diluted at a ratio of 1: 5 with TTBS solution (TBS with 0.1% Twin-20) and 1% BSA followed by incubation for 15 min at room temperature. The sites of nonspecific binding on Sepharose with the immobilized antigen were also blocked for 1 h in the same solution (TTBS containing 1% BSA). The phage suspension and 0.15 mL of Sepharose beads were placed into 15 mL centrifuge tube, which turned over overnight at 4 °C and then thoroughly washed with TTBS and, later, TBS. Phage particles bound to the resin were eluted with 0.75 mL 0.1 M trimethylamine for 5 min and the eluant was rapidly neutralized by addition of an equal volume of 1 M Tris-Cl (pH 7.5). The resultant phage suspension was added to 15 mL cell culture of *E. coli* XL1-Blue MRF⁺, that was grown up to OD_{600} 0.4 at 37 °C in the 2×YT medium. The mixture was incubated at the same temperature for 20 min without stirring, 50 min with stirring on an orbital shaker (260 rpm), bacteria were sown to ten dishes with a solid SOB-GA selective medium and colonies of infected cells were grown at 30 °C. Bacterial cells were scraped away with a glass spatula from the dishes and stored in 2×YT-GA with 25%

glycerol at -80 °C.

The infecting of 20 mL of these bacteria suspension in 2×YT-GA with OD_{600} 0.025 by the helper phage, its cultivation followed by PEG-precipitation of viruses, their incubation with an immobilized antigen, and the subsequent elution of viral particles enabled accomplishment of the second and the third rounds of the selection.

After the third round of panning the suspension of selected phages was additionally depleted against 0.3 mg of proteins from a homogenate of control (uninfected) locust fat bodies immobilized on 0.3 mL of CNBr-activated Sepharose 4B as described above. Sepharose incubated with the binding buffer instead of dialyzed proteins of uninfected fat bodies was used as a control. The sites of nonspecific binding were blocked in TBS containing 1% BSA for 1 h at room temperature. The resin was incubated in microcentrifuge tubes with 150 μl (1/10 part) of phage suspension after the third round of selection diluted 1:8 by TBS. After incubation on a mini-rotator overnight at 4 °C, resin-free viruses were used for infecting of bacteria and plasmid DNA production. PCR amplification of the sequences encoding selected recombinant antibodies was performed using primers pSEX Nco for (TGCTGCTGCTGGCAGCTCAG) and pSEX Not rev (TGATATCTTTGGATCCAG).

HETEROLOGOUS EXPRESSION OF SCFV FRAGMENTS IN BACTERIA *E. COLI*

The PCR-amplified pool of about 800 bp DNA fragments was excised from the gel and ligated into the pOPE101 expression vector at NcoI/NotI restriction sites. The obtained constructs were used for transformation of bacteria XL1-Blue MRF⁺ by electroporation. The colonies of transformants, which had been grown on dishes with the selective 2×YT-GA medium at 30 °C, were transferred as replicas onto nitrocellulose membrane and placed into the same, but glucose-free, medium containing 0.4 mM isopropyl- β -D-thiogalactopyranoside (IPTG, a specific inducer of a promoter controlling expression of antibodies). After 4-h incubation of the colonies in dishes at 30 °C, the membrane was boiled for 5 min in 0.5% SDS and used for Western-blot analysis with antibodies against a polyhistidine sequence (Merck, Germany) present in recombinant antibodies. Two hundred colonies exhibiting a high level of antibody expression were placed into wells of immunological multiwell plates and grown up to OD_{600} 0.4 in 2×YT-GA liquid medium. The cells

were harvested by centrifugation and resuspended in the same medium containing 0.04 mM IPTG but without glucose. After the expression of scFv antibodies over 4 h at room temperature, 1 mM PMSF, 1 mM EDTA-Na₂, and 1 µg/mL pepstatin A (final concentrations) were added to the suspension and bacteria were ultrasonically disrupted. Debris was precipitated by centrifugation of the plates at 2500 rpm for 30 min and the supernatant was used for testing the antigen-binding capacity of antibodies.

DOT BLOT ANALYSIS AND IMMUNOBLOTTING

Soluble fractions of homogenates of infected and control fat bodies of locusts were prepared like an antigen for CNBr-activated Sepharose. In the case of dot blot analysis two hundred small nitrocellulose bands with dots of these samples were placed into wells of immunological multiwell plates, blocked for 1 h in the presence of TTBS and 1% BSA and incubated overnight at 4 °C with supernatants of sonicated bacteria diluted 1 : 1 with the same blocking solution. For immunoblotting, a sample of infected fat bodies was mixed with the same volume of 125 mM Tris-Cl buffer (pH 6.8) containing 4% SDS, 10% 2-mercaptoethanol, and 20% glycerol followed by 10 min incubation at 95 °C. Proteins were separated by SDS-PAGE in 12% gel and transferred onto a nitrocellulose membrane by means of a blotting MiniTrans-Blot device (Bio-Rad, CA) according to its manual. The membranes were blocked for 1 h in the presence of TTBS and 1% BSA and incubated overnight at 4 °C with scFv antibodies (supernatant of sonicated bacteria) diluted 1: 30 with the same solution. Then, all membranes were washed in TTBS, incubated with monoclonal antibodies against polyhistidine sequence diluted 1: 2000 for 2 h at room temperature, washed again with TTBS and incubated with polyclonal antibodies against mouse immunoglobulins conjugated with horseradish peroxidase (Bio-Rad, CA) at the same conditions and dilution. After washing with TTBS and then TBS, the membranes were incubated to develop peroxidase reaction in the freshly prepared solution of TBS, 15% methanol, 0.05% 4-chloro-1-naphthol, and 0.02% H₂O₂.

Results

The described above approaches made it possible to construct a library of recombinant scFv-

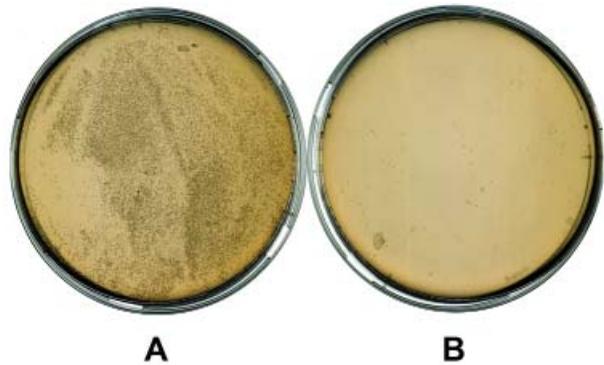


Fig. 1. The number of phages eluted from antigen-containing (A) and control (B) resins after the third round of selection. *E. coli* cells were infected by 1/500 volume of the suspension of eluted viruses, bacteria were plated on glucose/ampicillin containing SOB medium and incubated for 24 h at 30 °C.

antibodies containing approximately 10⁸ bacterial transformants with different combinations of VH and VL fragments of immunoglobulins. The number of bacteria taken for the first selection experiment was 2.5×10⁹ that exceeded the library's diversity by 25 times. Their infection with helper phage followed by cultivation under the conditions described above allowed to produce and isolate by PEG-precipitation approximately 10¹¹ infectious viruses. Incubation of produced phages with immobilized antigen and infection of bacteria by eluted material showed that one of about 300 000 virus particles bound with the immobilized antigen during the first round of selection. In the control experiment (Sepharose without antigen) the number of bound and eluted phages was four times lower. For the second and third rounds of panning the number of bacteria infected with helper phage was reduced 10 times. However, the number of viruses eluted from Sepharose and the ratio of phages eluted from antigen-containing resin over phages eluted from the control one significantly increased after the third round of selection (Fig. 1). It confirmed the specificity of scFv-antibodies exposed on phage surface against proteins extracted from the infected fat bodies of *L. migratoria*. Subsequent incubation of selected viruses with immobilized proteins of non-infected fat bodies decreased five times the number of phages in final suspension compared with control (Sepharose without proteins). It suggests that a portion of phages with antibodies specific to the host proteins was eliminated from the pool of selected viruses.

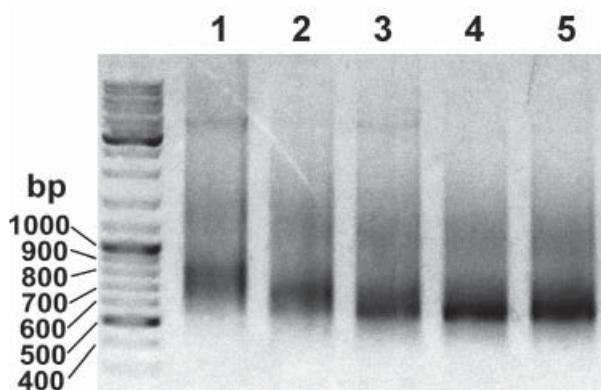


Fig. 2. PCR analysis of DNA fragments encoding scFv molecules showed accumulation of viruses with abnormally small antibodies during selection. *Lane 1* - amplification of 800 bp fragments encoding full-length scFv antibodies in the case of plasmid DNA of original library; *lanes 2-4* - gradual accumulating of phages with scFv genes of 550-600 bp took place from the first (*lane 2*) to the third (*lane 4*) round of selection; *lane 5* - depletion of the library against the proteins of uninfected fat bodies unaffected the presence of short variants in the phage population.

PCR-amplification of selected scFv-encoding sequences using primers pSEX Nco for and pSEX Not rev showed that as in the case of the first library (Dolgikh et al., 2017) accumulation of viruses with abnormally small size antibodies took place during the selection process. In the case of plasmid DNA of the original library, amplification of about 800 bp fragments corresponding to the genes encoding full-length scFv fragments was observed (Fig. 2, *lane 1*). However, phages with scFv genes of about 550-600 bp were gradually accumulating in the selected pools (Fig. 2, *lanes 2-4*). It is interesting to note that depletion of the library against the proteins of uninfected fat body did not affect the presence of short variants in the population of phages (Fig. 2, *lane 5*).

To analyze the antigen-binding activity of selected antibodies, scFv-encoding sequences were amplified using primers pSEX Nco for, pSEX Not rev and DNA fragments of about 800 bp were inserted into the pOPE101 expression vector. Their heterologous expression in *E. coli* allowed the search of scFv antibodies interacting with the proteins of the infected fat body.

Dot blot analysis of antigen-binding activities of scFv fragments produced by two hundred bacterial colonies revealed 3 transformants whose antibodies

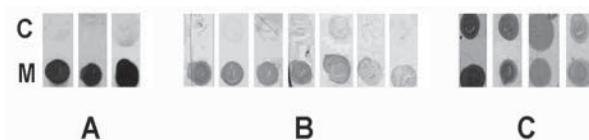


Fig. 3. Dot blot analysis of two hundred *E. coli* colonies transformed by pOPE101 expression vector with inserted genes of recombinant antibodies revealed three groups of bacteria, which produced active scFv fragments. A - Mini-antibodies of three transformants intensively stained proteins of infected but not control fat bodies; B - twenty transformants produced antibodies also specifically recognizing the proteins of the infected host tissue, but the staining intensity was much lower in this case; C - four bacterial colonies expressed scFv fragments equally staining proteins of infected and control fat bodies. *Abbreviations:* C- proteins of control (uninfected) fat bodies of the locusts; M- proteins of infected by microsporidia *P. locustae* fat bodies of the locusts.

specifically recognized and intensively stained protein samples of infected but not control locust fat bodies (Fig. 3, A). Twenty transformants produced antibodies, which also specifically recognized the proteins of the infected host tissue, but the intensity of staining, in this case, was not such high (Fig. 3, B). Here it should be emphasized that for preparation of samples the fat bodies of infected locusts were disrupted by glass homogenizer with a loose fitting Teflon pestle very gently in isotonic PBS, and microsporidia pelleted by centrifugation should be intact. Since parasite inner proteins were removed from the samples, the revealed scFv fragments should be specific to the proteins secreted by microsporidia into an infected cell or to the host proteins induced by infection.

In addition, four bacterial colonies expressed antibodies, which stained the samples of infected and control fat bodies. The intensity of coloration of both spots was similar for each antibody variant but differed between the transformants (Fig. 3, C). Since the infection with *P. locustae* did not affect the content of host proteins recognized by these antibodies, the last group of scFv fragments was out of scope of this study.

Immunoblotting of the samples from infected host tissue showed that three transformants of the first group produce scFv fragments recognizing the same protein of about 48 kDa (Fig. 4, *lane 1*). Nineteen bacterial transformants of the second

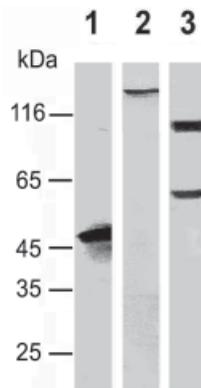


Fig. 4. Immunoblotting of samples obtained from the fat bodies of infected locusts showed that the first group of transformants (Fig. 3, A) produces scFv fragments recognizing the protein of about 48 kDa (lane 1). Nineteen bacterial transformants of the second group (Fig. 3, B) produced antibodies staining the high molecular weight protein of more than 120 kDa (lane 2). In addition, the scFv fragment of one bacterial colony in the latter group specifically recognized two bands corresponding to the proteins of about 63 and 90 kDa (lane 3).

group produced antibodies staining the high molecular weight protein of more than 120 kDa (Fig. 4, lane 2). In addition, the scFv fragment of one bacterial colony from this group specifically recognized two protein bands of about 63 and 90 kDa (Fig. 4, lane 3), which may correspond to two forms of *P. locustae* secretory a/b hydrolase found in previous studies (Senderskiy et al., 2014).

PCR-amplification of DNA fragment encoding the last mentioned antibody and its analysis with restriction enzymes HaeIII, MspI, MseI, and HindIII (Fig. 5, A) showed similar digestion pattern with the gene of anti-hydrolase scFv fragment selected in the previous study (Dolgikh et al., 2017). However, comparative analysis of nucleotide sequences encoding two scFv fragments revealed six differences in amino acid compositions of the VH fragments. The VL sequences differed in three amino acid residues (Fig. 5, B).

Discussion

The results of the study demonstrated that construction of scFv fragments in the form of highly representative libraries followed by their panning using phage display technology may be an effective

tool to search proteins involved in the interaction of microsporidia and other intracellular parasites with an infected host cell. After the screening of two hundred bacterial transformants, we have discovered mini-antibodies to several candidate proteins to begin their study. Further experiments with the library should discover new variants of recombinant antibodies interacting with parasite and host proteins.

Recombinant single-chain antibodies against proteins of intracellular parasites have been attracting the attention of researchers for more than a decade (Hoe et al., 2005; Wajanarogana et al., 2006; Abi-Ghanem et al., 2008). Many of the constructed scFv fragments demonstrated an ability to suppress the development of malaria plasmodium (Isaacs et al., 2011, Sumitani et al., 2013), cryptosporidia (Pokorny et al., 2008), trypanosomes (Ayub et al., 2012) and other intracellular parasites. Recently, the first data on suppressing of the intracellular development of entomopathogenic microsporidia by means of scFv-antibodies were published (Huang et al., 2018). In this study, we showed that scFv fragments can be used also to investigate the molecular machinery exploited by the intracellular protists to control physiological processes and molecular programs of an infected host cell. An important prerequisite for successful application of this approach is high representativeness of a constructed library of recombinant antibodies. The more VH/VL combinations will be obtained during its construction, the more antibodies to the proteins of interest can be selected using phage display technology.

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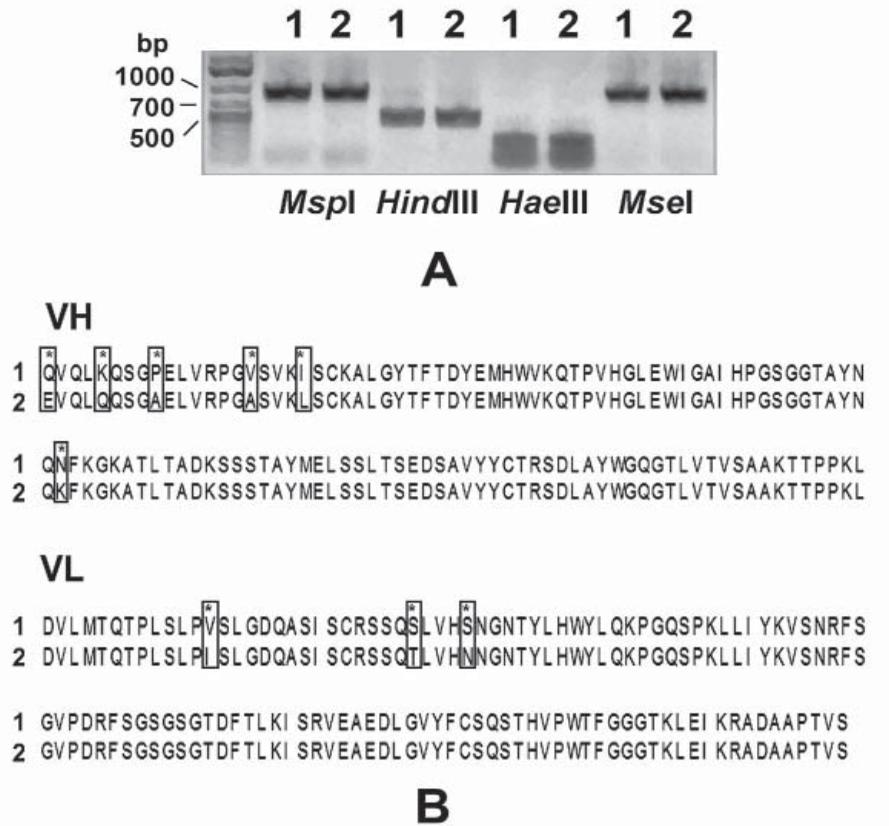


Fig. 5. Comparative analysis of sequences encoding (1) the new recombinant mini-antibody recognizing the proteins of about 63 kDa and 90 kDa from infected fat bodies of the locusts and (2) previously selected anti-hydrolase scFv fragment (Dolgikh et al, 2017). A - PCR-amplification of protein-encoding sequences followed by their incubation with four restriction enzymes showed similar digestion patterns; B - analysis of nucleotide sequences of two scFv fragments revealed some differences in their amino acid compositions (marked by the asterisks and enclosed in the squares).

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