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Construction, screening and identification of a phage display antibody library against the *Eimeria acervulina* merozoite

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ABSTRACT

A single-chain antibody library against *Eimeria acervulina* merozoites was constructed by phage display approach. Antibody-displaying phage was selected in four panning rounds against cryopreserved *E. acervulina* merozoites. Five clones were randomly selected from the fourth panning round, and their nucleotide sequences were aligned and compared to mouse germ-line sequences. Soluble antibody was produced in a non-suppressor *Escherichia coli* strain, purified by protein A affinity chromatography, and characterized by Western-blotting. Immunofluorescence assay showed localization of the produced recombinant antibody fragment on the surface *E. acervulina* merozoites. These resultant antibody fragments showed high specificity and binding capacity for soluble antigens and intact fixed merozoites which seems promising as diagnostic, therapeutic and/or vaccine tools against coccidiosis.

Introduction

Coccidiosis is a severe disease in chicken and recognized as a serious challenge for the poultry industry [1,2]. It is caused by several species of protozoan parasites belonging to the genus *Eimeria* [3,4]. *Eimeria acervulina* (*E. acervulina*) is one of the most pathogenic *Eimeria* species in terms of distribution, frequency, and economic losses. In most countries, prophylactic chemotherapy using ionophores and synthetic drugs is still the main method for the control of coccidiosis [5]. However, the long-term use of the preventative anti-coccidial drugs is implicated for the development of drug resistance [6,7] and accumulation of drug residues in livestock products [8,9]. Alternative strategies should be adopted to overcome such drawbacks. Recombinant protein vaccine production is a promising candidate for control of coccidiosis [10]. In this concept, coccidia immune protective antigen protein(s) should be identified, using antibodies with known specificity, to prepare single chain antibodies against chicken coccidiosis [9,11–13]. Yin et al. claimed that it is inevitable to attack coccidia by single-chain antibody–bacteriotoxin conjugates, namely, recombinant immunotoxin [14].

The initiation of antibody library technology, especially phage display is a promising tool for production of single-chain antibodies [15–17]. In this study, constructed phage antibody library was panned out using *E. acervulina* merozoites. Single-chain fragment variable antibody (ScFv) was selected and characterized by SDS–PAGE, Western-blotting, ELISA and immunofluorescence assays.

Materials and methods

Strains and reagents. E. acervulina Baoding strain was provided by Laboratory of Parasitology, College of Animal Science and Technology, Agricultural University of Hebei, China. *Escherichia coli* strain TG1 was provided by Tiangen Biotech Company Limited (Beijing, China). *E. coli* HB2151, Helper phage $M_{13}KO_7$, and HRP-anti M_{13}IgG were purchased from Pharmacia Corporation (USA). FITC-conjugated goat anti-mouse monoclonal antibody was purchased from Sino-American Biotechnology Co. (Luoyang, China).

Preparation of E. acervulina merozoites' antigen. E. acervulina was propagated by passage through 2-week old broiler chicks. The chicks were inoculated orally with 1.0×10^4 of sporulated *E. acervulina* oocysts. Merozoites of the second generation were extracted at 96 h post inoculation and purified [18]. The purified merozoites were used freshly for preparation of soluble antigen or cryopreserved. The soluble antigen of purified merozoites was prepared

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using ultrasonic disruption followed by high-speed centrifugation. The protein content of the antigen was estimated spectrophotometrically, adjust to 2.72 mg/ml and stored −20 °C till use.

Phage display antibody library construction. The heavy chain (V_H) and light chain (V_L) antibody genes against *E. acervulina* merozoites were amplified by RT-PCR using the total RNA, extracted from the spleen cells of BALB/C mice immunized with the soluble antigen of merozoites, as template. The single-chain fragment variable (ScFv) genes of 773 bp with *Sfi*I and *Not*I restriction sites were amplified with splicing overlap extension-PCR (SOE-PCR) using V_L *Sfi*I primer: 5′-CCTTTCTATGCGGCCCAGCCGGCCCAGCCGGCC-3′ and V_H *Not*I primer: 5′-TCCGGATACGGCACCGGCGCACCTGCGGCCGC-3′. The primers were designed by Primer 6.0 software, based on the published sequences (accession No GU235986 to GU235990) of variable regions, V_L and V_H [19,20]. Purified products were ligated into phagemid pCANTAB5E. Recombinant phagemids were transformed into *E. coli* TG1 and superinfected by $M_{13}KO_7$ helper phage.

Panning and detection. The antibody library was taken through four rounds of panning on *E. acervulina* cryopreserved merozoites, as described by Abi-Ghanem et al. [21]. In brief, the library was precipitated by addition of 2 ml PEG/NaCl for 30 min on ice bath and collected by centrifugation. Merozoites coated ELISA plates were blocked with 3% Bovine serum albumin (BSA) in PBS and incubated with freshly diluted phage at 37 °C for 1 h. After five

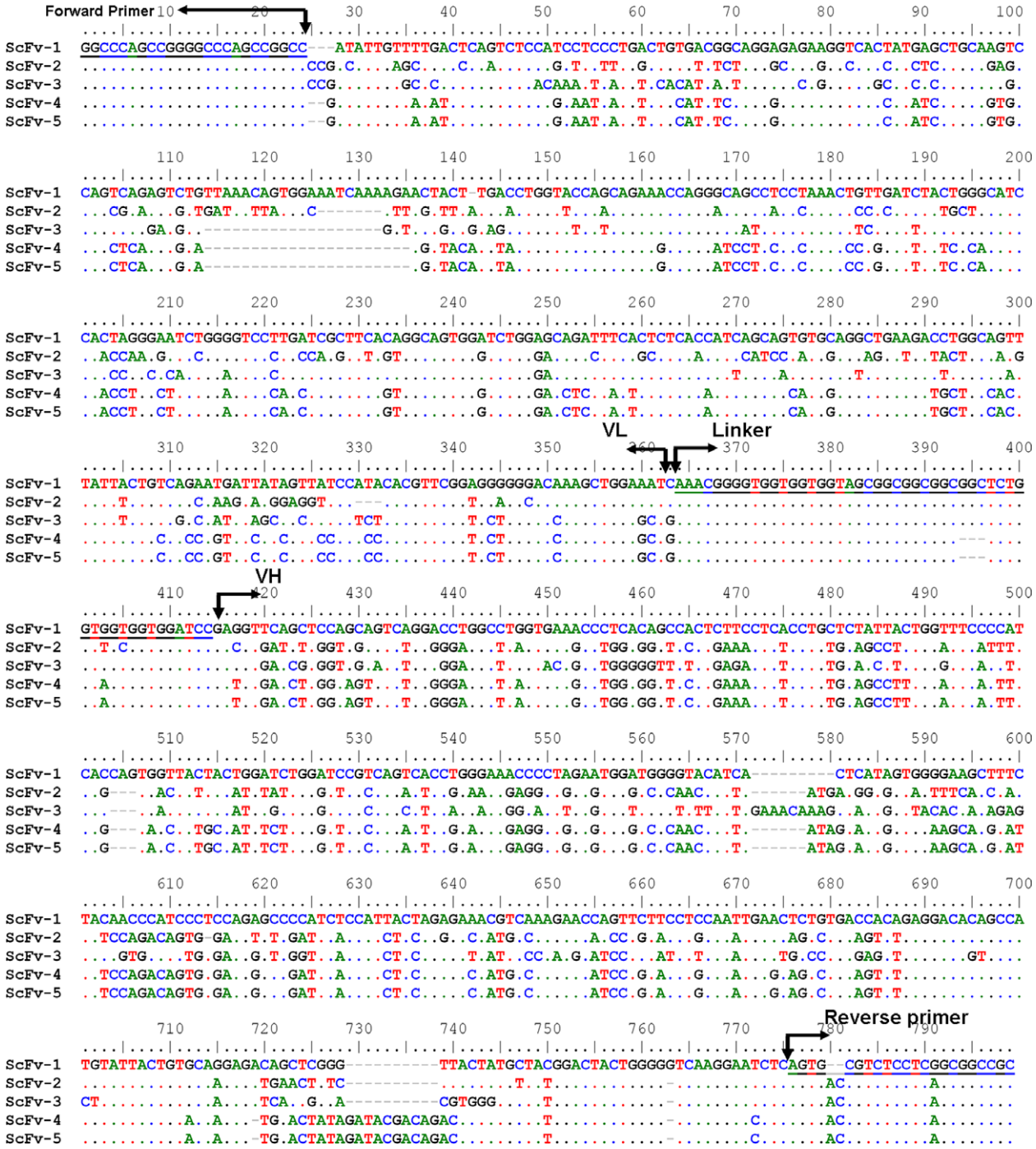

Fig. 1. The nucleotide sequence analysis of the five individual colonies of ScFv. Length of ScFv gene is about 773 bp, encoding 245 amino acids. V_L gene in the upstream fragment of about 320 bp in length, while V_H gene in the downstream fragment of about 370 bp in length. Length of linker sequence is 51 bp.