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ORIGINAL PAPER

Construction of scFv phage display library with hapten-specific repertories and characterization of anti-ivermectin fragment isolated from the library

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Abstract A specific phage display library was developed for screening antibodies against micro-molecular substances with 16-membered macrocyclic backbone. Through mRNA extraction, RT-PCR and gene splicing by overlap extension PCR (SOE-PCR), single-chain fragment variable (scFv) gene fragments about 750 bp were generated and ligated with the phagemid pCANTAB5E and were transformed into competent cells. A phage display library containing 2.4×10^6 clones was constructed from milbemycin oxime-bovine serum albumin (MILO-BSA) immunized mice. The screening was carried out by ivermectin-bovine serum albumin (IVM-BSA) with different concentration levels. Furthermore, scFv phage clones were isolated within the four round library panning and screening by phage- ELISA, 10 positive clones were obtained finally. These positive clones were then sequenced, and their soluble type antibodies were identified and showed significant binding activity.

Keywords Phage display library · scFv · 16-membered macrocyclic backbone micro-molecular substances · Screening

Introduction

Immunoassay is a good alternative method to replace instrumental analysis for the determination of pesticide or veterinary residues in various samples, which is based on the specific and reversibility of the reaction between the antigen and the antibody. Due to the high sensitive characters, trace components residue detection can be reached by immunoassay method, thereby what kind of sensitivity it going to be mostly depended on the antibody characters. Antibodies producing by different kinds of technologies influence the detection results. Polyclonal antibody technology and monoclonal antibody technology are traditional antibody producing methods. Commercial immunoassays kits still mostly made up by polyclonal antibodies and monoclonal antibodies so far.

Recombinant antibodies represent the next generation of immunochemical reagents extending options of polyclonal and monoclonal antibodies for application to clinical, environmental and food analysis [1–3]. The molecular biology tools such as vectors [4] and phage display libraries [5, 6] have been developed and used for the preparation of the recombinant antibodies against both protein antigens and small hapten structures. Phage display technology is a high-performance, time saving and effective technique for producing specific antibodies and has been widely used to produce antibodies. This technique is based on recombinant DNA methods to allow co-selection of recombinant antibodies and their respective genes [7]. Fab or scFv can be produced quickly and cheaply from phage display antibody libraries [8] without extra steps for antigen purification and needs of specialized equipment. Furthermore, it can be mass expressed in bacterial avoiding animal immunization. The generation of recombinant single-chain antibodies from either non-immune or immune phage display antibody libraries is another effective means to obtain high-affinity antibodies against a specific target, thereby permitting rapid screening and selection for high-avidity or tight binding scFv fragments with targeted specificity. Furthermore, due to the advantage of phage display technology with direct linkage between an observed phenotype and its encapsulated

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