

Recombinant antibody and its application in plant pathogens diagnosis

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Introduction

ELISA (Enzyme Linked Immunosorbent Assay) tests are routinely used for the detection of plant pathogens because of the specificity and rapidity of this diagnosis technology. These ELISA tests rely commonly on conventional antibodies. In opposite recombinant antibodies are artificial constructions produced by genetic engineering and one of the most remarkable molecules of these kinds are scFvs (single chain Fragment variable). ScFvs are made by the association of the variable heavy and light chain region and keep the binding properties of classical antibody (cf fig. 1). The use of recombinant antibodies brings some improvements that are described in this poster.

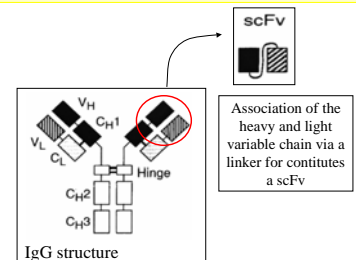


Figure 1: scFv structure

Principle

ScFvs can be made from an hybridoma line producing a monoclonal antibody but also in combination with the phage display technology¹, directly from an immunized animal (cf fig. 2) by passing the time consuming hybridoma production. Moreover, the step of animal immunization can be avoided by the use of a synthetic library (non immune library).

To select specific scFvs, the Phage display is used. It consists of displaying at the surface of phage a library of scFvs. The selection procedure is described in figure 3.

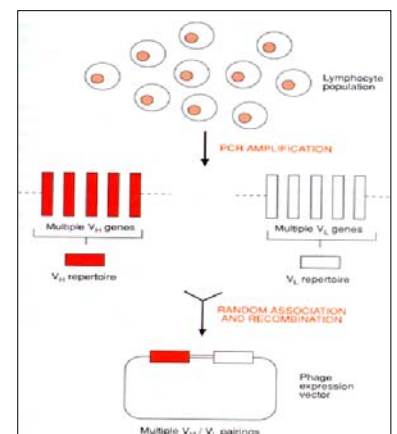


Figure 2: Making of an scFv library from immunized animal⁴

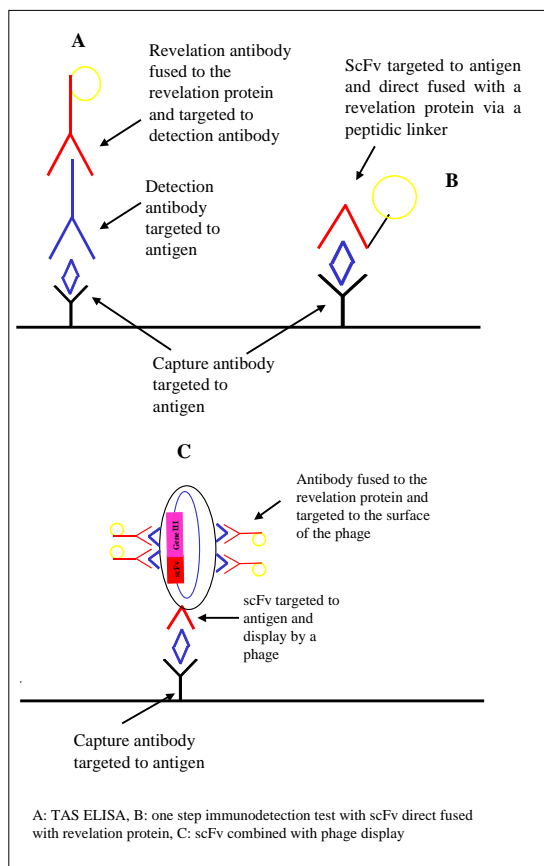


Figure 3: Principle of different immunodetection tests

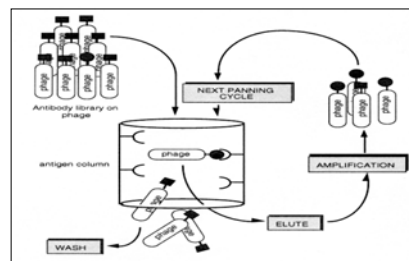


Figure 3: The Phage display¹

Routinely Elisa tests are indirect triple antibody sandwich (TAS). They require the use of a capture antibody and detection antibody that is specific of an antigen and a revelation antibody targeted to the detection antibody and linked to a revelation protein like alkaline phosphatase (cf fig. 3.A). Monoclonal recombinant antibodies selected by Phage display may be used in a one step immunodetection test if the gene encoding the scFv is directly fused with the gene encoding the revelation protein². In this case, it is not necessary to use a revelation antibody that binds to a constant fragment of the detection antibody (cf fig. 3.B). This is a real economy of reagent and time. More over, the lack of chemical fusing used for TAS between the antibody and the revelation enzyme avoids antibody degradation possibly caused by this process.

In order to amplify the detection signal, scFvs can be used in combination with phage. This is due to the fact that the antibody phage complex can be revealed by a conjugated enzyme antibody which binds a multiple epitope on the phage (cf fig. 3.C).

These recombinant monoclonal antibodies directly fused with a revelation enzyme may be also easily produced in bacterial or yeast expression system at low costs unlike the monoclonal antiserum. The classical antibody production from hybridoma cells requires different steps of chromatography purification which are not necessary with a bacterial expression system. The recombinant antibodies are produced in the periplasm of the bacteria or yeast and can be directly extracted and purified³.

Conclusions

The use of monoclonal recombinant antibodies is a new strategy for developing improved immunodetection tests for plant pathogens. This technology presents several advantages, facility and rapidity of use, sensibility and low cost production compared to the conventional antibody strategy.

Acknowledgments and references

¹Kay K. B., Winter *et al.*, 1996. Phage Display of Peptides and protein. San Diego: Academic Press, Inc. 344 p

²Remko A. *et al.*, 1999., Application of Phage display in selecting *Tomato spotted wilt virus* Specific Single-Chain Antibodies (scFvs) for sensitive diagnosis in Elisa. The American Phytopathology Society. 90(2), 183-190

³Liu. J. L. *et al.*, 2003. A vector system for the production of single-chain-Fc fusions in *Pichia pastoris* as detection reagents *in vitro*. Journal of Biochemistry. 134 (6), 911-917

⁴Roitt I., *et al.*, 2001. Immunology. Edimburgh: Mosby. 480 p