AFPTEST: Standard test kits based on novel recombinant antibodies for detection of harmful plant viruses

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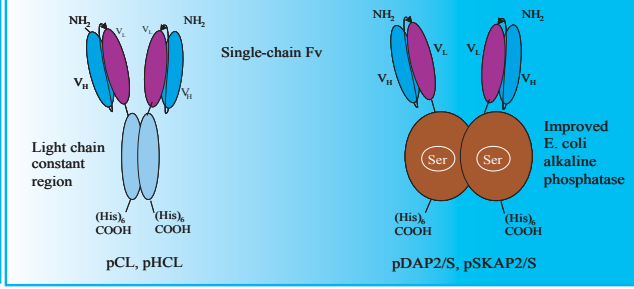
Introduction

Antibody fragments against a wide range of proteins can be obtained from genetic libraries of antibody genes using phage display technology. We have obtained specific antibody fragments from phage display libraries and cloned them into standard casettes for expression as fusion proteins (AFP) for use in serological assays.

The aims of this project were to demonstrate that such reagents were robust and compare them with existing enzyme-linked immunoassays (EIA) based on immune sera for virus detection. The virsues were beet necrotic yellow vein (BNYVV), potato leafroll (PLRV and tomato spotted wilt (TSWV).

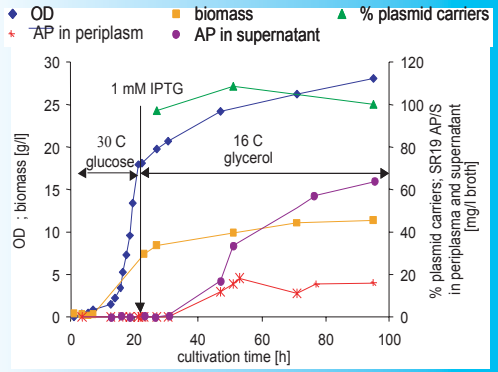
Three different systems (E. coli, Drosophila and Pichia pastoris) were compared for AFP expression, conditions for stability on storage and assay optimisation were determined and assays were optimised and validated in different laboratories.

Antibody fusion proteins - AFP

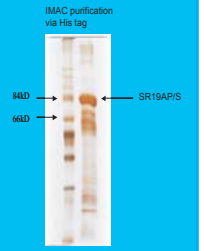


In vitro expression and purification

E. coli was found to be the best expression system and the plasmids were redesigned to use kanamycin or tetracycline as selectable markers instead of ampicillin to decrease plasmid loss during fermentation. Lower induction temperatures gave best results. Yields of up to 80mg/litre active protein.



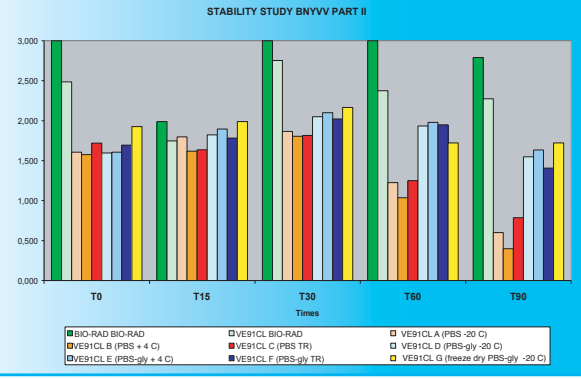
Fermentation of AFP SR19AP/S



Stability on storage

In the first tests we found that the AFP AP proteins lost activity within a few days of preparation. Therefore it was necessary to investigate different storage conditions and buffer additives to preserve activity.

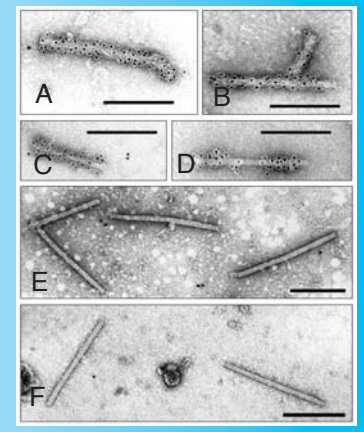
We found that lyophilisation for long term storage and dilution of AFP AP in buffer containing glycerol were effective in preserving activity of both coating and detecting reagents for BNYVV and PLRV for >3 months (the maximum period tested).



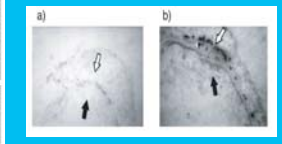
Assay optimisation and validation

Fully recombinant enzyme assays were devised using AFP HC_L to coat microtitre plates and AFP AP to detect target virus. The four AFP selected for PLRV and BNYVV detection produced stable and robust assays and were also suitable for use in tissue print and electron microscope immunogold labelling assays. In contrast, the TWSV AFP produced were not stable and were not suitable for further development. It is likely that the sequence of the single scFv sequence used to create the TSWV AFP conferred the i instability.

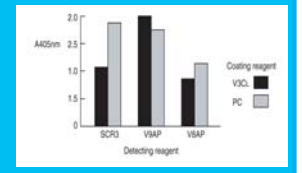
A commercial licence has been obtained to exploit the assays and suitable business opportunities are being explored.



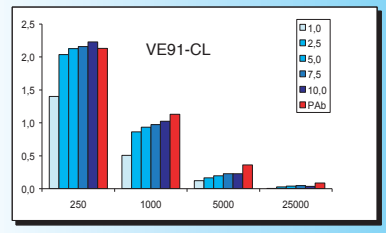
Immunogold labelling of BNYVV Ju2Petra particles from *C. quinoa*. A: decoration with scFv SR19 (diluted 1:10), detection with anti-C-*myc* mAb (diluted 1:50) and GAM-10 nm gold conjugate. **B:** Decoration with scFv SR19-AP (diluted 1:10), detection with anti-his mAb (diluted 1:100 Verd_nnt) and GAM-10 nm gold conjugate. **C:** Decoration with scFv VE91 (diluted 1:10), Detection with anti-C-*myc* mAb (diluted 1:100) and GAM-10 nm gold conjugate. **D:** Decoration with scFv VE91-C_L (diluted 1:10), Detection with anti-his mAb (diluted 1:100) and GAM-10 nm gold conjugate. **E:** negative control: primary Ab: anti-C-*myc* mAb (diluted 1:100), secondary mAb: GAM-10 nm (diluted 1:100). **F:** negative control: primary Ab: anti-his_6 mAb, secondary mAb: GAM-10 nm. bar = 200 nm



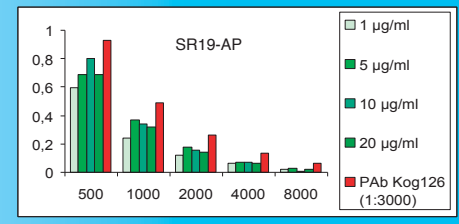
Tissue print immunoassay to detect PLRV in vascular tissues. a = non-infected control. b = infected, note dark black spots in vascular tissue. Arrows indicate position of internal and external phloem



Detection of PLRV in fully recombinant EIA; comparison with assay incorporating antisera (PC) and monoclonal antibody (SCR3) together with anti-mouse-AP.



Absorbance values obtained in EIA to detect BNYVV
Recombinant reagent VE91-CL is as effective as antiserum (Pab)



Absorbance values obtained in EIA to detect BNYVV
Recombinant reagent SR19-AP is as effective as antiserum (Pab)