ENGINEERING SPECIFIC PROBES AGAINST PLANT POLYSACCHARIDES USING PHAGE DISPLAY

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During the past ten years, immunological probes have proven to be very efficient tools in the study of plant cell walls. Antibodies have revealed temporal, spatial and developmental variations in the composition of cell wall polysaccharides (Liners *et al.*, 1994), demonstrating all the complexity of the organization of the wall phase, often considered in the past as a rigid and static structure.

Despite their usefulness, the collection of monoclonal antibodies against plant cell wall macromolecules and especially polysaccharides is very limited. In fact, downsides of the hybridoma technology such as poor immunogenicity of plant polysaccharides that results in low number of clones available for selection as well as the instability of some of these clones, make the production of polysaccharide-specific monoclonal antibodies still a tough challenge.

The recombinant antibody phage display approach has recently been developed to exploit the extremely large diversity of the mammalian immune system, increasing thus the probability to isolate more rare probes. Antibody phage display needs fusing the coding sequences of antibody variable regions to the sequence of a bacteriophage coat protein. The antibody fragments (scFv) are then presented on the phage surface. A selection step (panning) allows to isolate the phages that display antibody fragments specific for a given antigen (Hoogenboom, 1997). Large amounts of soluble fragments can easily be obtained by infecting bacterial cells with phages from antigen-positive clones.

We are generating a pool of recombinant antibody probes directed against polysaccharidic epitopes. Mice have been hyperimmunized with different polymers such as for example pectins, gum arabic, guar gums, alginates, carrageenans.... After polysera analysis by ELISA, the spleens of the animals have been taken and processed for the construction of a phage display library. Spleen mRNAs have been isolated and used for cDNA preparation. The PCR amplification of antibody variable region sequences has been carried out on cDNA, using primers complementary to the ends of the heavy- and light- chain variable region genes (VH-VL). Complete scFv genes have been obtained by PCR using a linker gene encoding a $(gly_4ser)_3$ peptide. A last PCR step has allowed amplifying scFv by flanking primers containing restriction enzyme sites available for cloning. We are now cloning these scFv genes into a phagemid vector to transform *E coli* TG1 cells and generate phage display library.

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References

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