HemiPop

Engineering structure and properties of poplar hemicelluloses

Project Start Month: January 2008

Project Duration: 36 months

Project Consortium

Project Coordinator

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Project Partners

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KTH, Associate professor Harry Brumer, AlbaNova University Center, SE-10691, Stockholm, Sweden

Project Objectives

The aim is to develop novel analytical and biotechnological tools for breeding of high value trees. The project will focus on hemicelluloses, in particular xylan in poplar, and contributes to the objectives of the WoodWisdom-Net programme by 1) resulting in new valuable know-how on the contribution of hemicelluloses to cell wall properties in poplar and by 2) developing and implementing new techniques for characterization of wood fibers.

Project Approach

The project will use poplar because it is a valuable model tree species for forest biotechnology. Poplar whole genome has recently been sequenced and the carbohydrate active enzymes, which are of particular interest for this project, have been identified. The project targets especially on xylan, the main hemicellulose component in poplar (23 % of dw), whose biosythesis and modification has attracted sofar less interest than cellulose and lignin.

Application of marker assisted breeding or transgene technology in the production of high value trees requires evaluation of the impact of key genes and enzymes in wood fibre biosynthesis. While gene expression can presently be relatively easily altered, the necessary chemical analysis of the secondary wall remains a major bottleneck and obstacle for rapid progress. The project will employ a combination of chemical, enzymatic and immunological technology with advanced analytical chemistry to develop new approaches that can be used to unravel the native xylan structure as well as the modified hemicellulose structures in transgenic trees. This will result in a link a specific change in xylan structure to the specific gene alteration.

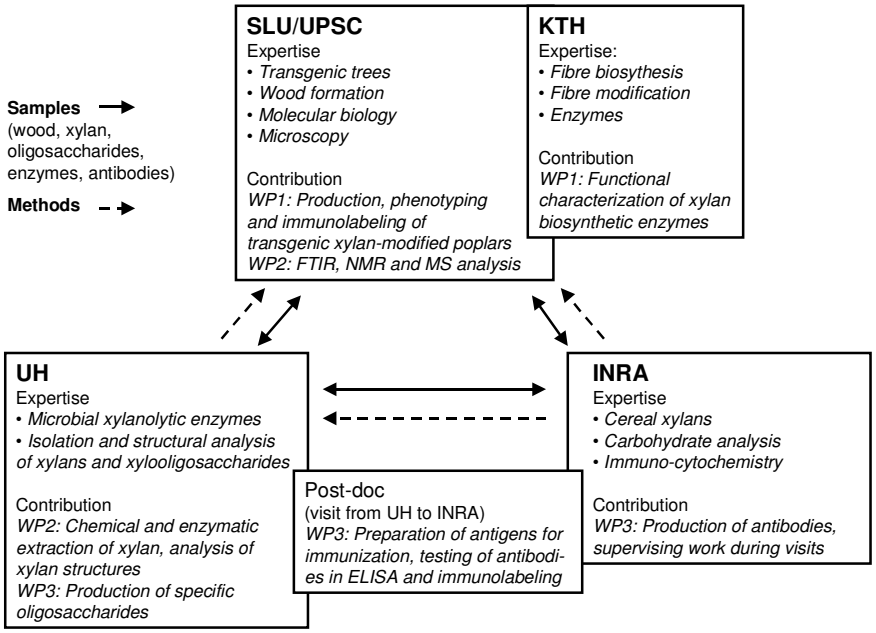
The project is organised in three workpackages:

WP1. Production of transgenic poplars with modified fiber chemistry (responsible SLU/UPSC)
SLU/UPSC and KTH have already identified a number of poplar carbohydrate active enzymes and other unknown function genes that are likely involved in secondary wall biosyntheses and modification. Microbial genes encoding xylan-acting enzymes to affect xylan content and/or to modify xylan structure in transgenic poplar lines will also be used for transgenic xylan modification in poplar. In addition to production of modified lines using constitutive promoter, an inducible system will be developed which will allow a more precise analysis of genetic modifications. Transgenic lines

will be produced in place at SLU/UPSC. Lines showing interesting phenotypes will be further analyzed in collaboration using analytical and immunological tools developed within the project (WP2 and WP3).

WP2. Cell wall analysis combining chemistry and enzymology (responsible University of Helsinki)
Suitable extraction method for poplar xylan will be developed. The extracted samples will be further purified, if needed, by physical, chemical or enzymatic methods. Methods for analysis of chemical composition, structure and size will be developed with the available advanced chromatography and spectroscopy techniques. Degree of polymerisation of xylans will be evaluated by HPSEC. Pure hydrolytic enzymes will be utilized for direct selective hydrolysis of xylan from wood samples for structural fingerprinting using chromatography and MS-techniques. The development of selective extraction and analysis methods will be carried out with wild type poplar samples. The established methods will be used for detailed structual analysis of xylans in interesting transgenig poplar lines in WP1.

WP3. Immunoprofiling techniques (responsible INRA)
Mono- and polyclonal antibodies against non-substituted xylan backbone are available in INRA. The development of new antibodies against glucuronic acid and acetyl groups will be done jointly by INRA and the University of Helsinki. The characterisation of the specificity of the antibody will be carried out using ELISA using a collection of oligosaccharides from various xylans and the antibodies will tested on wood samples using microscopy. The development of immunoprofiling techniques and *in situ* characterisation will be first carried out with wild type poplar samples after which the interesting transgenic poplar lines will be studied in WP1.



Expected Project Impact

The project will generate valuable data and fundamental information on the contribution of hemicelluloses (xylan) to cell wall properties in poplar. It will also result in new techniques for characterization of wood cell walls and it will contribute to our understanding of xylan biosynthesis in trees and the plasticity of the cell wall matrix. The information obtained will form the basis for tree breeding in order to engineer industrially valuable wood properties such as wood fiber strength and stiffness, improving pulping properties and saccharifacation for growing short-rotation high-energy crops of the future. The wood, pulp and paper, and future biorefining industry will benefit from the project

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