FibreSurf - New biotechnological tools for wood fibre modification and analysis

FINAL REPORT

Title of the research project	**FibreSurf - New biotechnological tools for wood fibre modification and analysis**
Coordinator of the project	Prof. Harry Brumer

BASIC PROJECT DATA

Project period	01-01-2008 - 31-12-2010
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URL of the project	-

FUNDING

Total budget in EUR	726 000 EUR
Public funding from WoodWisdom-Net Research Programme:	Total funding granted in EUR by source:
Finland	
Tekes - Finnish Funding Agency for Technology and Innovation	-
Ministry of Agriculture and Forestry (MMM)	-
Academy of Finland (AKA)	210 000
Denmark	
Danish Forest and Nature Agency (DFNA)	-
Danish Research Council for Production and Technology Sciences (FTP)	-
Germany	
Federal Ministry of Education and Research (BMBF)/	-

Project Management Agency Jülich (PtJ)	
Norway	
The Research Council of Norway (RCN)	-
Innovation Norway (INVANOR)	-
Sweden	
The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas)	198 000
Swedish Governmental Agency for Innovation Systems (VINNOVA)	-
France	
Ministry of Agriculture, General Direction for Forest and Rural Affairs (DGPAAT)	-
Technical Centre for Wood and Furniture (CTBA)	-
National Institute of Agronomical Research (INRA)	-
United Kingdom	
Forestry Commission (FC)	225 000
Nordic Forest Research Co-operation Committee (SNS)	-

Other public funding:

-

Other funding:

VTT, Finland	123 000

PROJECT TEAM (main participants)

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Elias Retulainen, Ph.D., Senior Research Scientist	M	Jyväskylä	VTT, Finland	Academy of Finland VTT
Antti Oksanen, M.Sc. Ph.D. student	M	Jyväskylä	VTT, Finland	Academy of Finland VTT
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Harry Gilbert, Ph.D., Professor	M	Inst. Cell and Mol. Biosci.	Univ. of Newcastle, UK	Forestry Commission
Lauren McKee, M.Sc., Ph.D. student	M	Inst. Cell and Mol. Biosci.	Univ. of Newcastle, UK	Forestry Commission
J. Paul Knox, Ph.D. Professor	M	Center for Plant Sciences	, Univ. of Leeds, UK	-
Gideon Davies, Ph.D. Professor	M	York Structural Biol. Lab.	Univ. of York, UK	-

DEGREES
Degrees earned or to be earned within this project.

2008	B.Sc.	M	Mr. Timo Rantanen	Univ. of Applied Sciences, Jyväskylä	
2011	Ph.D.	F	Ms. Lauren McKee, born 1985 MSc 2007 Environ. Biogeochemistry	Univ. of Newcastle	Prof. H. Gilbert, Univ. of Newcastle
2013	Ph.D.	M	Mr. Johan Larsbrink born 1982	KTH	Prof. H. Brumer KTH

ABSTRACT

The objective of this cross-disciplinary research initiative was to unite several unique approaches for the analysis and modification of wood fibres working toward new value-added materials and biofuel applications. New biotechnological tools were developed for the analysis, saccharification, and biomimetic application of xylans and xyloglucans, which are key hemicelluloses found in woody plants. Specifically, newly sequenced microbial genomes were mined for enzymes specific for the degradation of these polysaccharides to facilitate cell wall degradation and improve yields of feedstock sugars. Likewise, novel carbohydrate-binding modules (CBMs) were characterised and their specific affinities were exploited to generate new molecular probes to dissect wood cell wall ultrastructure and follow fiber modification. Finally, the ability to re-adsorb cell wall matrix polysaccharides, especially xyloglucan, onto cellulosic fibres was used to improve the surface and mechanical properties of both virgin and recycled wood pulps.

1.1 Introduction

1.1.1 Background

Describe the background of the project and the basic problem that it sought to address.

The exploitation of trees as a fibre resource or for liquid biofuels requires, on one hand, improvement of fibre surface interactions, and on the other, complete degradation of cell wall polysaccharides to constituent monosaccharides, which can then be utilized in microbial fermentations.

While much of the current focus within the bioenergy industry is on the utilization of cellulose-derived glucose, the ultimate economic viability of the process will require the exploitation of both C6 and C5 sugars (arabinose and xylose). The hemicellulosic polysaccharides xyloglucan (XG) and glucuronoarabinoxylan (GAX) cloak the cellulose microfibrils and must therefore be removed to increase the access of the cellulases for their substrate. Hemicelluloses can be removed by pretreatment with alkali, however, this process carries an economic and environmental penalty and thus the enzymic hydrolysis of these polymers is the preferred route. Unfortunately, the enzyme systems which remove the xylopyranose and arabinofuranose side chains of XG and GAX, respectively, are highly complex and our knowledge of these enzymes is presently poor. However, modern genomic sequencing provides a state-of-the-art approach to discover new microbial glycoside hydrolases and associated carbohydrate binding modules (CBMs) to begin address this problem.

In contrast to their degradation to provide monosaccharides, plant cell wall polysaccharides can find added value as strength-building agents in papermaking. XG in particular possesses a uniquely high binding affinity for cellulose fibres, has excellent solubility in water, and is readily available as an agricultural by-product (tamarind kernel powder, TKP). Wet-end addition of native XG added to pulp suspensions improves paper sheet stength properties, while spray application to paper sheets results in significantly greater improvements. In both cases, this can be directly attributed to XG-mediated cross-linking of fibre surfaces but very little is known about the nature of this interaction at the molecular level. Depending on localisation, XG has great

potential to affect the nature of interfibre bonds created during drying, and, consequently, the mechanical and rheological paper properties in the wet and dry web. The introduction of XG into fibre wall can also provide a new opportunity to reduce or prevent hornification and related inactivation of fibre surfaces, thereby improving the value of recycled fibre streams. Further, biochemical addition of functional groups to XG using enzymes such as xyloglucan endo-transglycosylase (XET) or galactose oxidase (GalOx) can improve fibre properties further through increased crosslinking.

1.1.2 Objectives

Describe the project objectives.

The project was divided into five major work packages, each focussed on either cell wall saccharification or cellulosic fibre improvement. Brief summaries of the goals of each work package are provided below.

WP 1: Discovery and characterisation of novel enzymes and binding modules that attack the sidechains of hard-and softwood xylans

The recent sequencing of several microbial genomes that display significant plant cell wall degrading activities have revealed a substantial expansion in genes encoding GH43 glycosyl hydrolases. For example, the genomes of *Bacteroides thetaiotaomicron,* a human colon symbiotic, and *Cellvibrio japonicus,* a soil saprophyte, contain 33 and 14 GH43 genes, respectively. To date GH43 contains relatively few enzyme activities that include arabinofuranosidases, arabinofuranosidases/xylosidases, arabinanases, xylosidases and a β1,3-galactanase. It is likely that several of the GH43 enzymes in *B. thetaiotaomicron* and *C. japonicus* are arabinofuranosidases that recognise the highly complex arabinofuranose-containing structures present in plant/tree arabinoxylan.

WP1a Expression and purification of recombinant GH43 enzymes.

Rather than expressing all 47 GH43 proteins from *B. thetaiotaomicron* and *C. japonicus*, we focussed on genes encoding enzymes that display similarity with known arabiofuranosidases or xylosidase/arabinofuranosidases.

WP1b Biochemical characterisation of GH43 enzymes.

To confirm that the GH43 enzymes display arabinofuranosidase activity, the capacity of these enzymes to hydrolyse aryl-glycosides and natural GAX oligosaccharides was assessed.

WP1c Structure determination of selected GH43 arabinofuranosidases.

To understand the mechanism by which the selected GH43 enzymes display novel substrate specificity requires 3D protein structural information, which was obtained by X-ray crystallography.

WP1d Identification and characterisation of CBMs that target the side chains of xylans.

It is likely that carbohydrate-binding modules which target the side chains of xylans will be linked to enzymes that attack the decorations appended to the backbone of this hemicellulose. The initial critical question was whether the CBMs bind to the backbone region of their cognate polysaccharides or whether they can exploit the side chains as specificity determinants.

WP2: Discovery and characterisation of novel enzymes involved in the systematic degradation of cell wall xyloglucans

To discover new enzymes involved in pathways of biological xyloglucan degradation, a combination of substrate synthesis, enzyme assays, and protein identification by mass spectrometry was employed.

WP2a. Expression, purification and characterisation of recombinant GH31 enzymes.

To date, α-xylosidase activity has only been observed in glycoside hydrolase family GH31. Our current analysis of the *B. thetaiotamicron* and *C. japonicus* genomes indicates 6 and 2 GH31 sequences, respectively. As GH31 also contains α-glucosidases, functional characterisation of these proteins is necessary to distinguish their specificities and applicability to aid XG saccharification. Following the strategy outlined in **WP1** above, selected sequences from these organisms in GH31 were cloned and expressed in *E. coli* for further enzyme analysis.

WP2b. Discovery of new isoprimeverose-releasing enzymes from C. japonicus. Isoprimeverase activity has been identified in crude fungal extracts long ago, but currently only one such enzyme has been purified to homogeneity. Unfortunately, the protein sequence of this enzyme is unknown, thus preventing the functional prediction of isoprimeverases in other microorganisms. Work in this WP, sought to indentify this activity in cultures of the soil saprophyte *C. japonicus* and correlate this activity with protein sequence from the recently completed genome of this organism.

WP2c. Determination of synergy between xyloglucanases and novel XGO-degrading enzymes.
To expand our knowledge of the xyloglucan degrading system of *C. japonicus*, an initial survey of the genome was performed to identify potential functionally homologous xyloglucanases from GH5 and 74, enzymes from which are highly selective for XG.

WP3: Saccharification of woody cell walls using newly discovered hemicellulases.

This WP formed a value-added contribution, based upon a nationally funded (UK BBSRC) project between the Newcastle and Leeds applicants on the use of carbohydrate-active enzymes to convert plant cell wall biomass to soluble sugars. In this project, tissues from taxonomically diverse plant species (tobacco stem, pea stem, celery petioles and maize coleoptiles) were digested with a variety of hemicellulases, which have been discovered and characterized in house.

WP4: XG-based modification of wood fibres for improved material properties.

This WP seeks to expand our fundamental understanding of the physical-chemical mechanisms of XG wood pulp modification and extend the application scope of this natural polymer as a paper additive.

WP4a. Effects of native XG on the properties of the consolidating and dry fibre network.
Using a specially designed spray rig, together with vacuum application, which mimics wet-web condition, XG was applied to paper sheets. The localisation of XG after this process was tested using XG binding proteins available within the consortium. Specifically, the effect of the molecular mass of XG on the penetration into fibre pores and accumulation at fibre-fibre joints will be tested with native (*M*w 500 000) and low mass (*M*w 50 000) XG was studied with specific XG-CBMs XG-mediated fibre crosslinking was expected to effect the dynamic strength and relaxation properties of the fibre network. A number of advanced techniques developed in-house were utilized to measure wet-web properties and induced stresses upon drying, including: layer analysis and splitting methods, dynamic strength and relaxation analyses for wet and dry paper, and measurement of the development of the uniaxial drying stress and strength with sheet dryness.

WP4b. The effect of new XG-based crosslinking chemistry on the properties of the fibre suspension, and on the properties of consolidating and dry fibre network. To further improve wet-web and dry sheet dynamic strength properties, cross-linking chemistry based upon the oxidation of the polysaccharide with the enzyme galactose oxidase (GalOx) was used. GalOx selectively oxidises the C-6 position of the galactose sidechains in XG to introduce aldehyde groups along the polysaccharide. These aldehydes are then capable of forming (hemi)acetals with hydroxy groups on other XG chains.

WP5: Novel XG- and XG/EXG-based systems to maintain or improve the properties of recycled wood fibre (A1,A2,A3,A5).

The ability of XG to penetrate into fibre pores and to coat fibre/sheet surfaces provides exciting possibilities to effect the properties of fibres which will be recycled into new products. In this WP, two aspects were specifically

addressed: fibre hornification, the stiffening and loss of bonding ability of fibres due to repeated drying and recycling; and de-inking, to remove tightly-bound, insoluble particles from fibre surfaces that degrade appearance.

WP5a. Effects of XG on fibre hornification. It is anticipated that such re-addition of a hemicellulose would effect the hornification and inactivation of fibre surfaces by mediating contacts between cellulose microfibrills in the fibre wall. Native high *M*w (500 000) low *M*w (50 000) XG were produced using xyloglucanases from the consortium project for adsorption onto chemical pulp surfaces. Hornification effects were tested by repeated sheet forming-drying-slushing cycles.

WP5b. Effects of XG on pulp de-inking. Our techniques for applying a protective layer of XG to fibre surfaces, together with the enzymological tools and expertise of the consortium partners, paves the way for a new system to facilitate the removal of particles during fibre recycling.

1.2 Results and discussion

Main achievements of the project, quality, innovativeness, industrial relevance and contribution to competitiveness, environmental and societal impact.

Specific, key results achieved thus far within the project are:

- KTH has cloned 7 novel enzymes predicted to be involved in hemicellulose degradation from glycoside hydrolase family GH31 into a recombinant expression host (*E. coli*). Recombinant expression, characterisation, and 3-D structure determination of one of these enzymes in nearly completed, and a manuscript is ready to be submitted in collaboration with the York group. Work on the other targets is ongoing.
    - **Larsbrink, J.** Izumi, A., Ibatullin, F., Nakhai, A., **Gilbert, H.J., Davies, G.J., Brumer, H.** (2011) Structural and enzymatic characterisation of a Glycoside Hydrolase Family 31 α xylosidase from *Cellvibrio japonicus*, submitted.
- KTH has developed and studied, in collaboration with Mats Ohlin, Lund Univ., a new, evolved carbohydrate binding module capable of detecting and locating xyloglucan in fiber walls.
    - Newcastle has hosted the KTH group to perform biophysical studies to elucidate the specificity of the novel xyloglucan-binding module (XGBM) for fiber applications. This work has lead to the publication of a manuscript in a peer-reviewed journal. Further, this XGBM has been employed by the VTT and Leeds group to visualize XG binding after fiber treatment (see below).
        - von Schantz, L., **Gullfot, F.,** Scheer, S., Filonova, L., Cicortas Gunnarsson, L., Flint, J.E., Daniel, G., Nordberg-Karlsson, E., **Brumer, H.,** and Ohlin, M.* (2009) Affinity maturation generates greatly improved xyloglucan-specific carbohydrate binding modules. *BMC Biotechnol.*, **9**, 92. DOI: 10.1186/1472-6750-9-92
    - Structural studies on this XGBM have also been performed and published by the KTH group:
        - **Gullfot, F.,** Tan, T.C., von Schantz, L., Nordberg Karlsson, E., Ohlin, M., **Brumer, H.,** and Divne, C.* (2010) The crystal structure of XG-34, an evolved xyloglucan-specific carbohydrate-binding module. *Proteins*, **78**, 785-789. DOI: 10.1002/prot.22642
- Newcastle has cloned all target glycoside hydrolase family GH43 targets from the soil saprophyte *Cellvibrio japonicus*. Work is ongoing to determine enzyme specificities and

kinetic parameters to understand the role of these enzymes in xylan degradation. In the course of these studies we have used HPLC, 1D and 2D NMR to show that one of the GH43s displays a novel arabinofuranosidases activity. The enzyme removes alpha1,2-arabinofuranose resides from single or doubly substituted backbone arabinose molecules. The crystal structure of the enzyme reveals a curve surface which can accommodate arabinan but not xylan chains. Specificity for the *O2* linkage is conferred by a hydrogen bond between an Asn and the endocyclic oxygen.

  - Cartmell, A., **McKee, L. S**., Peña, M. J., Larsbrink, J., Brumer, H., Lewis, R. J., Viksø-Nielsen, A., **Gilbert, H. J.** and Marles-Wright, J. (2011). The structure and function of an arabinan-specific alpha-1,2-arabinofuranosidase identified from screening the activities of bacterial GH43 glycoside hydrolases *J Biol Chem submitted*

- We have also determined the structure of a second GH43 enzyme that removes *O3* arabinofuranose sugars specificially from doubly substituted backbone sugars in arabinan and xylan. The structure reveals an extended substrate binding cleft where specificity is conferred through numerous interactions with the O2 linked arabinose backbone, while the orientation of the backbone is conferred by a hydrogen bond with the endocyclic oxygen of the +1 xylose. Significantly, removal of a tyrosine at the lip of the active site cleft introduced xylanase activity. Thus we have generated a single enzyme that displays both endo and exo side chain cleaving activities, which uses a common active site. Such an enzyme may have utility in the biofuel arena. Our enzyme discovery activity has also identified a xylanase that displays absolute specificity for arabinoxylan through specific intereactions with the O3 arabinose linked to the active site xylose. Such an activity has not previously been reported and, again the enzyme may have applications in the deconstruction of cereal-based arabinoxylans. The enzyme is linked to a novel CBM that, though calcium-mediated avidity effects, targets galactose-containing polysaccharides. Lauren McKee made a contribution to the work and is listed as an author in one of the back to back papers submitted to JBC.
  - Montanier, C. Y., Correia, M.A.S., Flint, J.E., Zhu, Y., **McKee, L.S.**, Prates, J.A.M., Polizzi, J., Coutinho, P.M., Henrissat, B., Fontes, C.M.G.A. and **Gilbert, H.J**. (2010) A novel non-catalytic Carbohydrate-Binding Modules displays specificity for galactose-containing polysaccharides through calcium-mediated oligomerization *J Biol Chem submitted*

- In addition, we have identified a GH43 arabinanase that appears to hydrolyze arabinan that is decorated with side chains; previous such enzymes are only reported to be active on unsubstituted arabinans. These enzymes have been deployed to study arabinan structure in plant cell walls in collaboration with Paul Knox, which has resulted in the following publication
  - Verhertbruggen, Y., Marcus, S. E., Haeger, A., Verhoef, R., Schols, H. A., McCleary, B. V., **McKee, L**., **Gilbert, H. J**., and **Knox, J. P.** (2009) Developmental complexity of arabinan polysaccharides and their processing in plant cell walls, *Plant J 59*, 413-425. DOI: 10.1111/j.1365-313X.2009.03876.x

- With respect to CBM work at Newcastle we have characterized, in collaboration with Paul Knox and gideon Davies, novel family 35 CBMs that recognize uronic acids in pectins or both pectins and xylans. The crystal structure of these proteins have revealed the mechanism by which these CBMs recognize their target ligands, and the role calcium plays in the process.

- Montanier, C., van Bueren, A. L., Dumon, C., Flint, J. E., Correia, M. A., Prates, J. A., Firbank, S. J., Lewis, R. J., Grondin, G. G., Ghinet, M. G., Gloster, T. M., Herve, C., **Knox, J. P.**, Talbot, B. G., Turkenburg, J. P., Kerovuo, J., Brzezinski, R., Fontes, C. M., **Davies, G. J.,** Boraston, A. B., and **Gilbert, H. J.** (2009) Evidence that family 35 carbohydrate binding modules display conserved specificity but divergent function, *Proc Natl Acad Sci U S A 106*, 3065-3070. DOI: 10.1073/pnas.0808972106

- We have also identified a CBM that specifically binds to xylose at the non-reducing end of polysaccharide chains
  - Abbott, D. W., Ficko-Blean, E., van Bueren, A. L., Rogowski, A., Cartmell, A., Coutinho, P. M., Henrissat, B., **Gilbert, H. J.**, and Boraston, A. B. (2009) Analysis of the structural and functional diversity of plant cell wall specific family 6 carbohydrate binding modules, *Biochemistry 48*, 10395-10404. DOI: 10.1021/bi9013424

- Additional novel CBMs that recognize levan, rhamnogalaturonan-I, xylan/cellulose/galactan, alphą or betą galactose and specifically alphą-galactose have been characterized both structurally and biochemically and will be the subject of publications in 2010.
  - Correia MA, Abbott DW, Gloster TM, Fernandes VO, Prates JA, Montanier C, Dumon C, Williamson MP, Tunnicliffe RB, Liu Z, Flint JE, Davies GJ, Henrissat B, Coutinho PM, Fontes CM, Gilbert HJ. (2010) Signature active site architectures illuminate the molecular basis for ligand specificity in family 35 carbohydrate binding module. *Biochemistry 49*, 6193-205.PMID: 20496884

- Finally we have shown that CBMs that bind to xylan or cellulose cause a siginificant enhancement in xylanase activity, mainly GH11 but also GH10, against intact cell walls. The data provide the first evidence that these non-catalytic modules contribute to the initial stages of the degradative process. The work also showed that the cellulose-directed CBMs caused a profound improvement in pectinase action against cell walls, while the xylan-specific CBMs enhanced the capacity of arabinofurnaosidases to remove the side chains from arabinoxylans in intact cereal cell walls. The data is not only relevant to industrial applications but also provide insight into the architecture of cell walls.
  - Hervé C, Rogowski A, Blake AW, Marcus SE, **Gilbert HJ, Knox JP.** (2010) Carbohydrate-binding modules promote the enzymatic deconstruction of intact plant cell walls by targeting and proximity effects. *Proc Natl Acad Sci U S A 107,* 15293-8. PMID: 20696902

- A related study between Knox and Gilbert showed that GH11 xylanases were less active against intact cell walls compared to GH10 xylansaes, while the reverse occurs when the two classes of enzyme are presented with purified xylans. These data provide a biological rationale for why organisms often produce both GH10 and GH11 xylanases.
  - Herve C, Rogowski A, Gilbert HJ and Knox JP (2009) Enzymatic treatments reveal differential capacities for xylan recognition and degradation in primary and secondary plant cell walls *Plant Journal 58,* 413-422

- VTT has performed an extensive set of experiments to determine the effect of the polysaccharide xyloglucan on the strength properties of the wet paper web. Compared with the papermaking standard, starch, xyloglucan addition has significant positive effects on runnability and other parameters. Combining cross-linking chemistry to XG treatments of fibres was noticed to enhance further wet strength properties of the web.

- VTT has started paper recycling studies with XG based treatments of fibre networks. Positive recyclability results were observed due to modifications of fibre surface with XG. Strength properties of recycled paper were further increased when XG treatments were combined with cross-linking chemistry.
- The VTT and Leeds groups have used the xyloglucan-binding module (characterised above by KTH and Newcastle) to locate xyloglucan in sheets following formation by fluorescence microscopy. This data provides a more detailed overview of the mechanisms responsible for the observed effects on papermaking.
    - All of the above results from the VTT group have been disseminated at a diversity of forest products-related conferences worldwide (see below).

1.3 Conclusions

The most important contributions to the state-of-the-art, derived from the results and discussion.

Work in this project has led to a number of contributions to the state-of-the-art along two lines. We have generated a large body of data on the enzymes and their associated binding modules, which degrade plant cell walls. This has significantly expanded the kit of biotechnological tools for fibre modification and analysis, which was one of the main goals in the project. Additionally, we have gained signification new insight into the application of exogenous polysaccharides, specifically xyloglucan, to improve fibre networks. Thus, native and modified XG significantly improve dynamic wet web strength, which is a key parameter affecting paper machine runnability. In addition to this and related technical advances, the project has successfully demonstrated that biotechnological tools, in the form of carbohydrate-binding modules, can be developed and employed to specifically localise polysaccharides in fiber matrices (e.g., paper). In sum, the project highlights the significant potential that exists in the union of biotechnology and forest products research.

1.4a Capabilities generated by the project

Knowledge generated in the project / outcomes of the project, such as unpublished doctoral theses, patents and patent applications, computer programs, prototypes, new processes and practices; established new businesses; potential to create new business opportunities in the sector.

The extensive amount of the fundamental information generated in this project on hemicellulose degradation will inform future studies that map enzymatic activity onto gene phylogenies. We have made a significant contribution to the knowledge contained in the Carbohydrate-Active Enzyme Database (www.cazy.org), which seeks to collect all such information to aid the functional assignment of glycosidase families. The modification of cellulosic fibers with the hemicellulose xyloglucan, both in native and enzyme-modified forms, has opened interesting new possibilities for papermaking (see also Section 1.4b).

1.4b Utilisation of results

Give a brief description of how the results of the research and development have been used and/or what is the exploitation plan or plans for transferring the results into practice.

While many of the outcomes of the project were fundamental in nature, the VTT (Technical Research Centre of Finland) partner was instrumental in developing the application of the polysaccharide xyloglucan as a paper wet-strength additive in laboratory and pilot scale applications. Some specific outcomes were the following:

Studies were focused in finding the effect of xyloglucan (XG) preparations on consolidating and wet or dry fibre networks. The addition took place either to pulp suspension or to wet fibre network by spraying. The effect of XG on never-dried and dried bleached birch pulp was studied. It was noticed that during pulp drying the fibre surfaces are inactivated (hornified), and this inactivation can be compensated for by a xyloglucan addition. Also, influence of XG based pre-treatment of fibre surface on the bonding ability of fibres after recycling was studied. Positive wet and dry strength improvements of samples were observed before and after recycling due to pre-modification of the fibre surface with XG. Additionally, XG layer on fibre surface was found to enhance deinkability of paper. Strength properties of paper were further increased by crosslinking XG with borax. The molecular weight of XG was found to have an essential role in the development of the strength of wet and dry paper. Further, enzymatically-oxidized XG (provided by KTH) was tested in paper making trials, in which it was found to increase substantially wet and dry strength of paper. Besides static laboratory tests, positive results were also noticed when XG-borax treatment was applied at dynamic semi-pilot environment. As a result of trans-national co-operation with British and Swedish partners a fluorescence based labelling method was applied in VTT Jyväskylä for locating XG in the fibre network. Microscopic images revealed that XG was on the surface of the fibre and no penetration to cell wall was observed.

In summary, these studies further support the positive impact of XG as an environmentally friendly papermaking chemical and comprise a step toward subsequent industrial utilisation.

1.5 Publications and communication

a) Scientific publications

For publications indicate a complete literature reference with all authors and for articles a complete name. Indicate the current stage of the publishing process when mentioning texts accepted for publication or in print. Abstracts are not reported. Indicate the five most important publications with an asterisk.

1. Articles in international scientific journals with peer review

Gilbert, H.J., Stålbrand, H., Brumer, H. (2008) How the walls come crumbling down: Recent structural biochemistry of plant polysaccharide degradation. Curr. Opin. Plant Biol., 11, 338-348. DOI: 10.1016/j.pbi.2008.03.004

von Schantz, L., Gullfot, F., Scheer, S., Filonova, L., Cicortas Gunnarsson, L., Flint, J.E., Daniel, G., Nordberg-Karlsson, E., Brumer, H., and Ohlin, M. (2009) Affinity maturation generates

greatly improved xyloglucan-specific carbohydrate binding modules. *BMC Biotechnol*., 9, 92. DOI: 10.1186/1472-6750-9-92

Gullfot, F., Tan, T.C., von Schantz, L., Nordberg Karlsson, E., Ohlin, M., Brumer, H., and Divne, C.* (2010) The crystal structure of XG-34, an evolved xyloglucan-specific carbohydrate-binding module. *Proteins*, 78, 785-789. DOI: 10.1002/prot.22642
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Verhertbruggen, Y., Marcus, S. E., Haeger, A., Verhoef, R., Schols, H. A., McCleary, B. V., McKee, L., Gilbert, H. J., and Knox, J. P. (2009) Developmental complexity of arabinan polysaccharides and their processing in plant cell walls, *Plant J 59*, 413-425. DOI: 10.1111/j.1365-313X.2009.03876.x

Montanier, C., van Bueren, A. L., Dumon, C., Flint, J. E., Correia, M. A., Prates, J. A., Firbank, S. J., Lewis, R. J., Grondin, G. G., Ghinet, M. G., Gloster, T. M., Herve, C., Knox, J. P., Talbot, B. G., Turkenburg, J. P., Kerovuo, J., Brzezinski, R., Fontes, C. M., Davies, G. J., Boraston, A. B., and Gilbert, H. J. (2009) Evidence that family 35 carbohydrate binding modules display conserved specificity but divergent function, *Proc Natl Acad Sci U S A 106*, 3065-3070. DOI: 10.1073/pnas.0808972106

Abbott, D. W., Ficko-Blean, E., van Bueren, A. L., Rogowski, A., Cartmell, A., Coutinho, P. M., Henrissat, B., Gilbert, H. J., and Boraston, A. B. (2009) Analysis of the structural and functional diversity of plant cell wall specific family 6 carbohydrate binding modules, *Biochemistry 48*, 10395-10404. DOI: 10.1021/bi9013424

Herve C, Rogowski A, Gilbert HJ and Knox JP (2009) Enzymatic treatments reveal differential capacities for xylan recognition and degradation in primary and secondary plant cell walls *Plant Journal 58,* 413-422

Hervé C, Rogowski A, Blake AW, Marcus SE, Gilbert HJ, Knox JP. (2010) Carbohydrate-binding modules promote the enzymatic deconstruction of intact plant cell walls by targeting and proximity effects. *Proc Natl Acad Sci U S A 107,* 15293-8. PMID: 20696902

Correia MA, Abbott DW, Gloster TM, Fernandes VO, Prates JA, Montanier C, Dumon C, Williamson MP, Tunnicliffe RB, Liu Z, Flint JE, Davies GJ, Henrissat B, Coutinho PM, Fontes CM, Gilbert HJ. (2010) Signature active site architectures illuminate the molecular basis for ligand specificity in family 35 carbohydrate binding module. *Biochemistry 49*, 6193-205.PMID: 20496884

Gilbert HJ (2010) The biochemistry and structural biology of plant cell wall deconstruction. *Plant Physiol* 153:444-455.

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Cartmell, A., McKee, L. S., Peña, M. J., Larsbrink, J., Brumer, H., Lewis, R. J., Viksø-Nielsen, A., Gilbert, H. J. and Marles-Wright, J. (2011). The structure and function of an arabinan-specific alpha-1,2-arabinofuranosidase identified from screening the activities of bacterial GH43 glycoside hydrolases *J Biol Chem submitted*

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2. Articles in international scientific compilation works and international scientific conference proceedings with peer review

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3. Articles in national scientific journals with peer review

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4. Articles in national scientific compilation works and national scientific conference proceedings with peer review

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5. Scientific monographs

Timo Rantanen (2008) Bachelor's Thesis, "Influences of Xylolglucan application of wet web characteristics and quality of paper", University of Applied Sciences, Jyväskylä

Lauren S. McKee (2011) PhD dissertation entitled "The functional diversity of GH43 enzymes" *to be submitted in March 2011.*

6. Other scientific publications, such as articles in scientific non-refereed journals and publications in university and institute series

a) Other dissemination
Such as text books, manuals, user guidelines, newspaper articles, TV and radio programmes, meetings and contacts for users and results.
Dissemination of results to industrial partners and industrial partners dissemination within the company.

Major conference presentations:

Elias Retulainen, International Austrian Paper Conference, 19-20 May 2010., Graz, Austria

Elias Retulainen, PTS Paper Symposium, 7-9 Sept 2010, Munich Germany

Antti Oksanen, 2010 TAPPI PEERS & 9th Research Forum on Recycling, 17-20 October 2010, Norfolk, Virginia / USA

Manu Somerkallio, Spray application of strength chemicals, October 2010, Tampere, Finland

Antti Oksanen, 4th International Symposium on Emerging Technologies of Pulping and Papermaking, 8-10 November 2010, Guangzhou / China

Brumer, H., Invited lecture, International Workshop on Wood Biorefinery and Tree Biotechnology, 21-23 June 2010, Örnsköldsvik, Sverige

Brumer, H., Invited lecture, Lignobiotech One Symposium, March 28th-April 1st 2009, Reims

Gilbert HJ, Invited lecture, Lignobiotech One Symposium, March 28th-April 1st 2009, Reims
Brumer, H., Invited lecture Plant Polysaccharide and Applied Glycoscience Workshop 29-31 July 2010, Tokyo, Japan

Larsbrink, J., Poster presentation, Plant Polysaccharide and Applied Glycoscience Workshop, 29-31 July 2010, Tokyo, Japan

Brumer, H., Invited lecture, Umeå Plant Science Center lecture series, 25 January 2010 , Umeå, Sweden

1.6 National and international cooperation

Give a brief description of the cooperation/ networking (partnership between the project participants and how this has developed; industrial involvement; synergies of industrial and research expertise; Has the project collaborated with similar projects in the WW-Net countries or other regions, or established new links with/ between local or international organisations involved in the respective research field? Describe how these partnerships have supported the project.
National vs. transnational aspects in the project; added value for the project and its impacts which result from transnational cooperation.

The project has, for the most part, been steered through bi-lateral discussions between the groups involved in specific sub-projects. As the main sub-projects are between the coordinator and the individual partners in Finland and the UK, decision processes have gone smoothly. The coordinator has maintained an active flow of information between the partners, thus keeping the various partners informed of progress in different areas.

As is evidenced by the number of joint publications and summary of unpublished work above, the trans-national aspects of this project developed well. In particular, new contacts were established between the Leeds and VTT groups in the area of fibre microscopy. Existing contacts and partnerships from previous joint projects were further strengthened by a dynamic exchange of research materials and new ideas.

The coordinator was also active in the HemiPop project, coordinated by Prof. Tenkanen (Univ. Helsinki) and involving partners from the Umeå Plant Science Center, Sweden, and INRA, France. This interaction opened the possibility to exchange experimental materials and expertise related to hemicellulose enzymology and analysis. Enzymes and carbohydrate-binding modules from FibreSurf are available to HemiPop researchers, while specific polysaccharide fragments (useful as enzyme substrates) are available to FibreSurf from HemiPop.