SUNLIBB

Sustainable Liquid Biofuels from Biomass Biorefining

Grant Agreement no. 251132

Collaborative Project
EU 7th Framework Programme
ENERGY

Project duration: 1st October 2010 – 30th September 2014

Deliverable 3.5

"Identification of activity of 3 new enzyme activities"

Authors: **Dr. Mathias Sorieul (University of Cambridge)**
Prof. Paul Dupree (University of Cambridge)

Workpackage: **3**
Workpackage Leader: **Prof. Paul Dupree (University of Cambridge)**

Due date: **March 2013 (Project Month 30)**
Actual submission date: **September 2013**
Revised version submitted: **December 2014**

Dissemination Level: **PU**

This project has received funding from the European Union's Seventh Programme for research, technological development and demonstration under grant agreement number 251132

SUNLIBB deliverables

Del No: D3.5	**Deliverable Name: D3.5 Identification of activity of three new enzyme activities**			
WP3	**Lead partner:** P9 UCAM	**Dissemination level:** PU	**Delivery date : month 30 31/3/13**	**Actual delivery date:** 31/3/13

Objective:

The aim was to describe the enzymatic activities of at least 3 genes involved in the elaboration of the polysaccharide matrix. This deliverable relates to task 3.3. This work focuses on enzymes involved in the xylan biosynthesis machinery, because this is the main non-cellulosic polysaccharide that impacts on the recalcitrance of biomass of Miscanthus, maize and sugarcane. The genes are related in different organisms, so discovering the activity in one species provides a high quality prediction for genes of interest in the other crops.

Results:

Despite the importance of grass arabinoxylan (AX), especially in biofuels, the enzymes responsible for the addition of the arabinosyl substitutions were unknown. We aimed to decipher which enzymes were responsible for this process. Some clues led us to investigate the poorly characterised GT61 family.

The wheat endosperm of the TaXAT1 RNAi line was analysed by high-performance anion-exchange chromatography (HPAEC) and ^{1}H-NMR. Those techniques showed a specific decrease in Araf α-(1,3)–linked to mono-substituted Xylp in AX. These results in transgenic wheat strongly suggest that GT61 family members of Clade A are responsible for arabinosylation of xylan.

To analyze the biochemical activity of GT61s in an *in vivo* system without intrinsic arabinosyltransferase activity, we chose heterologous expression of epitope-tagged wheat TaXAT1 and TaXAT2 in the stem of the Arabidopsis *gux1 gux2* double mutant (*gux*), which lacks glucuronosyl substitutions in stem xylan. Analysis by capillary normal-phase liquid chromatography (NP-LC) followed by MALDI-ToF-MS and MALDI-ToF/ToF-MS/MS showed that XA^{3}XX oligosaccharide was present.
Moreover two Arabidopsis FOX lines expressing rice OsXAT2 and OsXAT3 (TaXAT2 homologs), were analysed. PACE fingerprint and MS analysis showed the presence of XU^{4m2}XXA^{3}XX. This therefore indicates that these rice GT61 enzymes similarly direct the addition of α-(1,3)–linked Araf to the xylan backbone
These results strongly suggest that grass XATs in GT61 Clade A possess xylan α-(1,3)-arabinosyltransferase activity. Therefore, P9 UCAM showed that two wheat genes and two rice genes (TaXAT1, TaXAT2, OsXAT2, OsXAT3) from the GT61 family are involved in the addition of α-(1,3)-arabinosyl residues onto the xylan backbone (Anders *et al*., 2012).

This finding demonstrates the plasticity of xylan structure *in planta*. We have demonstrated a profound alteration of the properties of AX, so manipulation of GTs activity promises to allow tailoring of AX biosynthesis to specific end-uses.

The GT61 maize and sugar cane orthologues are provided in D3.4.

A second activity relates to the glucuronic acid side chain. Previously, glucuronic acid side chains have been shown to be important in xylan extractability from biomass, and P9 had identified the gene encoding these enzymes in Arabidopsis. These GlcA residues carry a 4-O-methyl ether, but the function and enzymes adding this structure were unknown at the start of this project.

The activities of four DUF579 genes have been investigated. The enzyme activities of two were tested by establishing an *in vitro* assay of methyl transfer onto xylan. Secondly, knock out mutants were generated in Arabidopsis, including a triple mutant. These experiments have shown that the enzymes are adding the 4-O-methyl group onto the glucuronic acid residues on xylan. Therefore they are glucuronoxylan methyltransferases. The genes are: At1g33800, At4g09990, At1g09610, At1g71690 and form the Arabidopsis GXM family.

The GXM maize and sugar cane orthologues are provided in D3.4.

We therefore have identified the activity of 4 enzymes of the GT61 family from rice and wheat and from 4 GXM enzymes from *Arabidopsis.* The maize and sugar cane orthologues have been identified and added to the list in D3.4. D3.5 is thus achieved.

The activities of Arabidopsis IRX9, IRX14, IRX10 and IRX15 plus related genes and DUF288, which are involved in the xylan backbone and cellulose synthesis respectively, were also investigated.

Our soon-to-be-published results demonstrate that IRX9 has mainly a structural role, while IRX14 has a functional DxD motif, which indicates a possible active catalytic site. Moreover some pull-down assays indicate that the GT43 (IRX9-L and IRX14) are forming a complex and interacting with other xylan synthesis enzymes. These data give an overview of the mechanisms involved in the biosynthesis of the xylan backbone.

The IRX maize and sugar cane members (orthologues of the GT43 (IRX9 plus IRX14) and GT47 (IRX10)) are described in the D3.4. The catalytic residues are conserved, and so the functions discovered can be reasonably assigned to the grass orthologues.

The collaborative work done for the characterisation of DUF288, led to the discovery of the involvement of this poorly characterised family in cellulose deposition and crystallinity. This work is going to be published soon.

Discussion /Conclusion:

Since the activities of 8 new enzymes (4 GT61 and 4 GXM) were characterised in xylan synthesis, the deliverable has been fulfilled. We also know more about the role of xylan backbone synthesis enzymes IRX9, IRX14 in grasses. This work is now leading to a new series of investigations.

Anders N, Wilkinson MD, Lovegrove A, Freeman J, Tryfona T, Pellny TK, Weimar T, Mortimer JC, Stott K, Baker JM, Defoin-Platel M, Shewry PR, Dupree P, Mitchell RA. PNAS **109** (3) 989-93