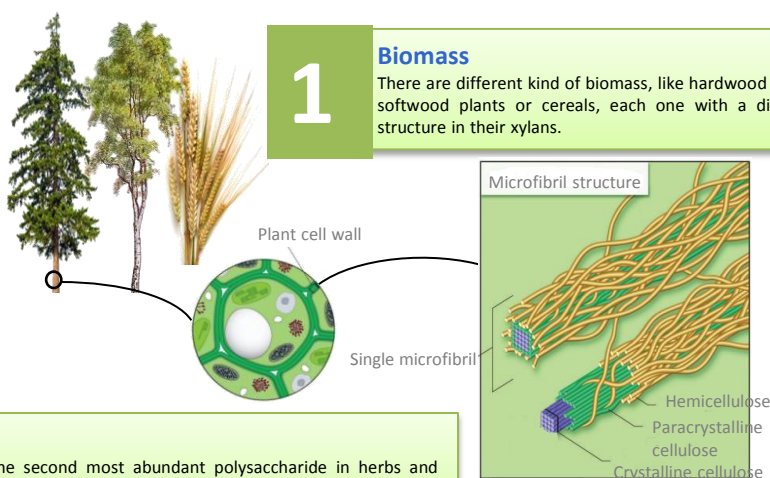


The use of materials from renewable resources is attaining increased importance, and the industries are seeking to replace petrochemical-based feedstocks with products derived from natural biomass, representing an abundant, inexpensive and readily available resource of renewable lignocellulosic biomass. Understanding the fine **intramolecular substitution pattern** of xylans is therefore crucial to elucidate efficient ways to exploit these huge bioresources. In this sense, the high specificity of **glycoside hydrolases** make them powerful tools in the structural characterization and targeted modification of hemicelluloses.

1

There are different kind of biomass, like hardwood plants, softwood plants or cereals, each one with a different structure in their xylans.



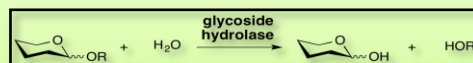
2

Xylan, whose structure differs from plant to plant, is the second most abundant polysaccharide in herbs and hardwoods, demanding the collaboration of a group of enzymes during their degradation.

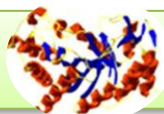


3

Multiple enzymes, including **endo- β -xylanase** (EC 3.2.1.8), **β -xylosidase** (EC 3.2.1.37), **α -glucuronidase** (EC 3.2.1.139), **α -L-arabinofuranosidase** (EC 3.2.1.55), and **acetylxyylan esterase** (EC 3.1.1.6) act synergistically in the deconstruction of xylans in plant cell walls. We have focused in **glycoside hydrolases**, a type of enzymes that catalyze the hydrolysis of the glycosidic linkage of glycosides. Glycoside hydrolases can catalyze the hydrolysis of O-, N- and S-linked glycosides. Specifically, the **endo- β -xylanase Family 10 and Family 11** (EC 3.2.1.8) are based on endohydrolysis of (1 \rightarrow 4)-**D**-xylosidic linkages in xylans .



4



Polypeptide chain and assumes (α/β)8 TIM-barrel fold, which consists of eight major parallel β -strands arranged side-by-side forming a cylinder in the center followed by eight major α helices.

Collins T., Gerday C., Feller G. (2005). *Xylanases, xylanase families and extremophilic xylanases*. FEMS microbiology Rev; 29(1):3-23.

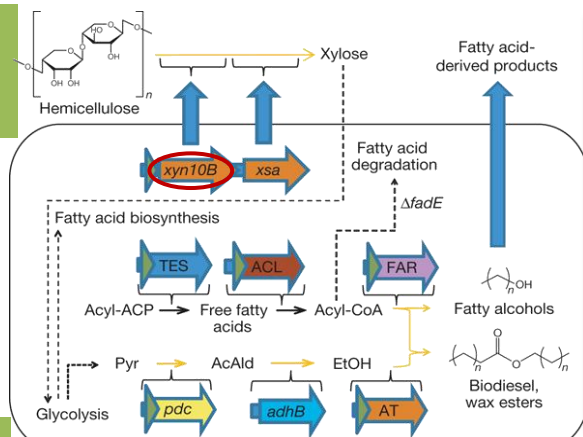
5



Globular protein composed of two β -sheets. Outer sheet A has five antiparallel β -strands, enriched with polar and uncharged serine and threonine amino acids, respectively. Sheet B is composed of nine antiparallel β -strands with front side forming active site, whereas the rear side of the sheet binds with sheet A forming hydrophobic core of the enzyme.

Collins T., Gerday C., Feller G. (2005). *Xylanases, xylanase families and extremophilic xylanases*. FEMS microbiology Rev: 29(1): 3-23.

6



Steen E.J., Kang Y., Bokinsky G. (2010). *Microbial production of fatty-acid-derived fuels and chemicals from plant biomass*. Nature: 463(7280): 559-562.

Based on amino acid sequence similarity, catalytic domains of xylanases can be substantially divided into two groups, **families 10 and 11 catalytic domains of glycoside hydrolases**. CBMs, most of which bind preferentially to cellulose, are also classified into families on the basis of amino acid sequence similarity. In addition, some xylanases contain other functional domains.

The *Clostridium stercorarium* xylanase Xyn10B is a modular enzyme comprising two thermostabilizing domains, a family 10 catalytic domain of glycosyl hydrolases, a family 9 carbohydrate-binding module (CBM), and two S-layer homologous (SLH) domains.

The production of advanced fuels and chemicals such as FAEs and fatty alcohols from sugar has a promising advantage over first generation products, such as corn ethanol and plant oil-derived biodiesel. To further simplify the FAE process, the genes encoding an endoxylanase catalytic domain (**Xyn10B**) from *Clostridium stercorarium* and a xylanase (**Xsa**) from *Bacteroides ovatus* were engineered into the FAE-producing *E. coli*. To hydrolyse the hemicellulose in the growth medium into xylose, which could then be imported and catabolized using the native *E. coli* metabolism, the hemicellulases were fused on their amino termini to the *E. coli* **protein OsmY**.

7 CONCLUSIONS

- ✓ The mechanism by which **endo- β -1,4-xylanases** are able to hydrolyze a range of decorated xylans remains unclear. Target bioinformatics studies of the expanding sequence and structure databases for new GH structures and families could be important for outstanding research opportunities that could significantly advance understanding for improving lignocellulose deconstruction.
- ✓ In recent years, the expanded availability of ethanol from corn starch and of biodiesel from soybeans has supplemented the transportation fuel supply but raised concerns regarding competition between biofuel production and the food supply.
- ✓ The type, number, and cost of glycoside hydrolase (GH) enzyme mixtures required for polysaccharide hydrolysis have been problematic for large scale deployment at commercial biorefineries. GH enzyme mixtures remain one of the largest costs to biofuels production.
- ✓ The expression of the **endoxylanase xyn10B** from *Clostridium stercorarium* and the **xylanase xsa** from *Bacteroides ovatus* as chimeras with OsmY allowed *E. coli* to grow on xylan as a sole carbon source.