Review article

Plant biomass degradation supported by non-enzymatic proteins

Rafał Madaj,[1*] Halina Kalinowska,[2] Elżbieta Sobiecka[1]

[1] Institute of General Food Chemistry, Lodz University of Technology, Stefanowskiego 4/10, 90-924 Lodz
[2] Institute of Technical Biochemistry, Lodz University of Technology, Stefanowskiego 4/10, 90-924 Lodz

*rafal.madaj@dokt.p.lodz.pl

Received: 09 February 2017/Available on-line: 24 April 2017

Abstract: *Lignocellulosic biomass, rich in potential carbon sources and value added products, has been intensively investigated in scope of its cost-efficient and effective decomposition. Many methods were developed, physicochemical or biological. Nevertheless, they are either expensive, inefficient or pose threat to the environment. Recently discovered proteins, lacking any hydrolytic activity, can be a key to solve problems associated with a slow process of enzymatical, eco-friendly degradation. These proteins belong to three related groups – swollenins, expansins and loosenins. Using different molecular mechanisms, they disrupt hydrogen bonds within cellulose chains, enabling enzymes to perform hydrolysis leading to decomposition of lignocellulosic complex.*

Keywords: *cellulose, enzymatic hydrolysis, swollenins, expansins, loosenins.*

Introduction

The demand for products derived from the renewable resources increase every year. Vast majority of waste by-products obtained - especially plant biomass-derived – voids any application apart from combustion or biofuel synthesis. Nevertheless, plant-based biomass, in order to be used otherwise than combusted, require pretreatment leading to simplifying its structure.

The most eco-friendly and cost-efficient way to degrade plant-based material is biodecomposition. Lignocellulosic biomass has been increasingly utilized in the production of fuels and chemicals, as an alternative to fossil fuels [1, 2, 3]. One of major bottlenecks of its conversion into glucose and other simple sugars is the need of efficient pretreatment to expose cellulose and hemicellulose chains to subsequent enzymatic degradation [4, 5].Enzymatic degradation of plant wastes, especially cellulose contained in plant cell walls, may be accelerated using certain specific proteins that weaken or disrupt hydrogen bonds between cellulose chains. Moreover, lack of part of these proteins would make plant

growth significantly impeded. These proteins encompass expansins, swollenins and loosenins, produced by microorganisms or plants. Their structure and function will be presented within this paper.

Lignocellulose components

Plant biomass is rich in lignocellulose complex. It consists from polymers like polysaccharides (mainly cellulose and hemicellulose) and aromatic alcohols (lignin) [6, 7]. Both of aforementioned constituents are essential for its mechanical recalcitrance and chemical durability.

Cellulose

Being the most abundant polysaccharide in nature[8] cellulose is the principal structural component of algal and plant cell walls. Its plenitude makes it being one of predominant renewable source of glucose for biochemistry and biotechnology [9]. It consists from β-D-glucopyranose residues connected via β-1,4-glycosidic bonds, forming polymers up to 16,000 units. Its chains, regardless the origin, create crystalline, rod-like structures known as microfibrils [8, 10]. The spatial arrangement of chains in microfibrils is responsible for its durability. Parallel cellulose chains form sheets that stack vertically and are staggered by half the length of a glucose residue.

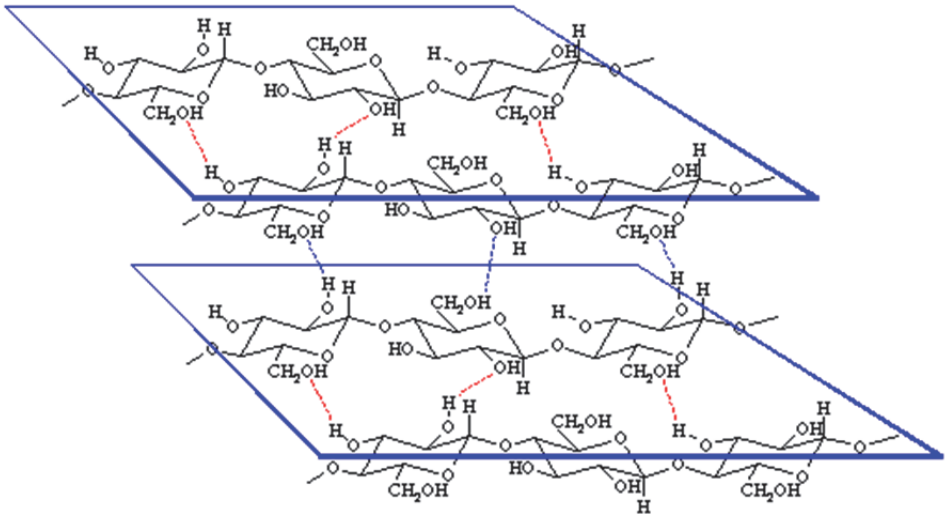

Figure 1. Arrangement of cellulose chains forming microfibrils [8]

Spatial structure of cellulose fibers encourages forming intra- and intermolecular hydrogen bond formation. The more exposed they are to the fiber surface, the more prone they are to disruption.

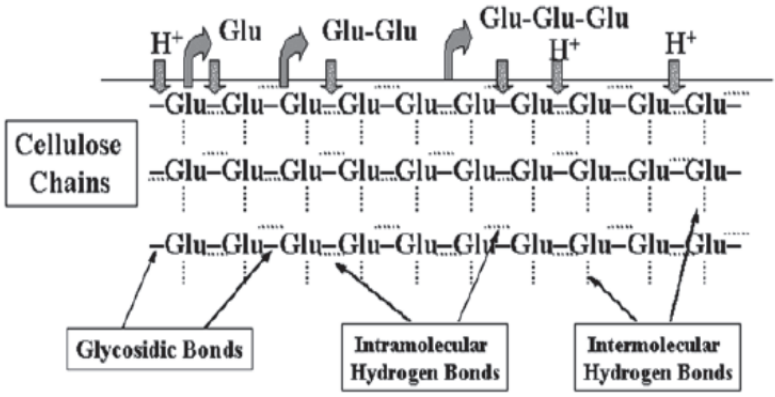

Figure 2. Hydrogen bonds within cellulose fibers [11]

Cellulose macrofibers form crystalline and amorphous regions. While the former consists of regular, three dimensional structure provided by hydrogen bonds, the latter is irregular and contain another substances like hemicelluloses or other polymers [12]. In plant cell walls, the presence of hemicelluloses and lignin within free spaces between cellulose fibers contributes to the stability of cellulose structure and resistance against the attack of specific enzymes catalyzing degradation of these polymers.

Hemicellulose

Hemicellulose is a branched structure made of many polysaccharides, with the most abundant xylans, arabinans, galactans and mannans. Unlike cellulose, its polymers are much shorter, therefore does not form many hydrogen bonds what results in weaker resistance to degradation [13].
Hemicellulose is strictly connected to lignin and cellulose, constituting in whole complex's durability.

Lignin

Lignin forms a recalcitrant layer made of phenylpropane units. It consists of three aromatic alcohols – sinapyl, coniferyl and paracoumaryl – forming a complex, cross-linked , polymeric structure [14] (Figure 3).

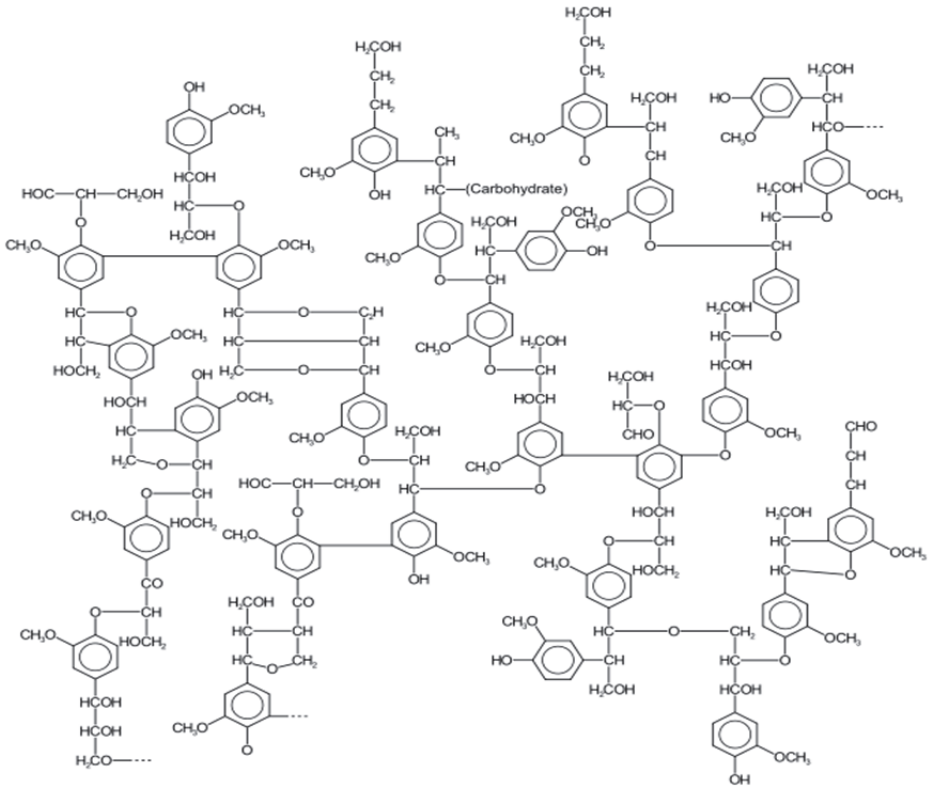

Figure 3. An exemplary structure of lignin [13]

The durability of lignin lies in number of ether bonds between monomers, supported by hydrogen bonding. Because of this structure lignin is the most resistant to biodegradation. It is responsible for rigidity and mechanical strength of plants. It renders plant cell walls resistant to water and pathogens. It fills the free spaces between fibrils of cellulose and other polymers [14].

The main difference compared to other components of cell wall lies in structure of lignin, which differs among plant species. Due to that, lignins were categorized into hardwood, softwood and grass-based.

Methods of lignocellulose pretreatment

There is a vast number of pretreatment techniques providing decomposition of lignocellulose complex to sole components, as shown on figure 4. Their main idea is to obtain a good carbon source and/or value added product [14, 15].

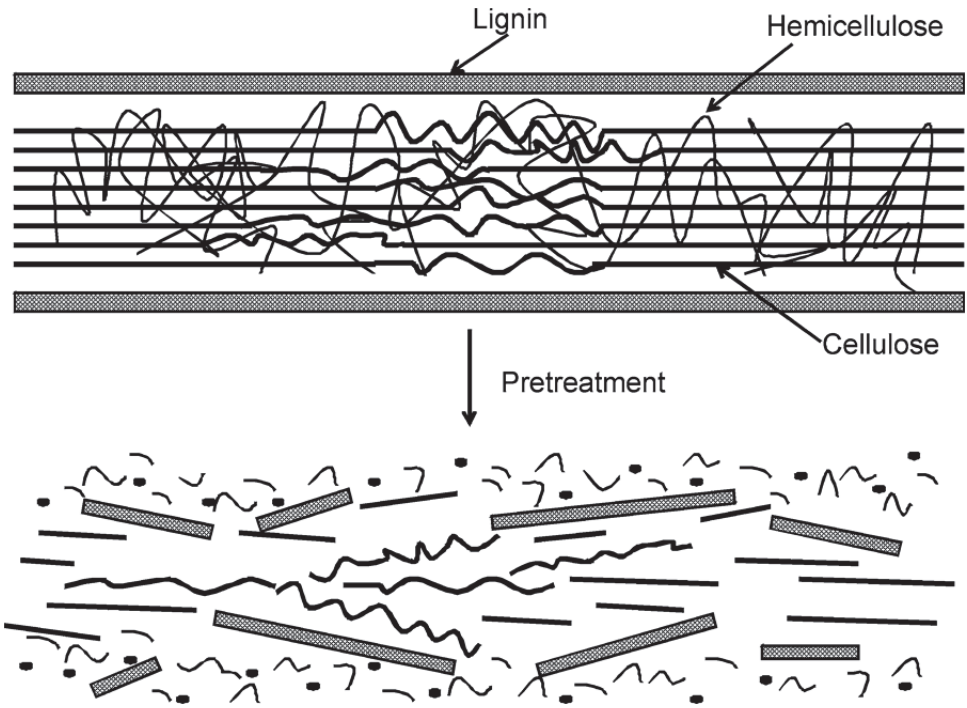

Figure 4. An effect of pretreatment on lignocelluloses [16]

Physicochemical methods

These methods base on purely physical methods like chipping, smashing, grinding, milling or – most efficient – heating via microwave radiation with combination of chemical pretreatment using acidic or basic environment. Nevertheless, these methods are either very expensive or inefficient, pose serious threat to environment and provide fermentation inhibitors [15, 16].

Biological methods

In contrary to methods described above, biological methods are eco-friendly and utilize either whole organisms or enzymatic preparation. As physicochemical methods can be toxic or impede further processing like fermentation, biological pretreatment would be a natural choice. Nevertheless, it requires to maintain sterility to evade contamination as well as pH, for optimal enzymatic activity. Moreover, using whole organisms result in biomass consumption, what eliminates such process from industrial-scale appliance. Therefore, industrial-scale appliance can only be found for enzymatic preparations, despite their decent efficiency.

However, recently-discovered proteins have no enzymatic activity, but are able to facilitate enzymatic degradation of lignocellulosic structure. Therefore they may have huge impact on biological pretreatment of biomass when

combined with enzyme preparations. These proteins were distinguished into three types: swollenins, expansins and loosenins.

Proteins participation in cellulose degradation

The process of plant growth and thickening is controlled by a group of specific proteins. They may be divided into two groups - hormones, existing in a single or bound with ligand, being directly responsible for growth, structure and appearance of plant fruits, and proteins lacking enzymatic activity, simplifying the process of growth. There are three wide groups of such proteins – expansins, swollenins and loosenins [16, 17].

Expansins

These proteins are responsible for extension of cell walls by loosening it, during maturing of plant cell walls, as the environment inside the cells is slightly acidic, optimal for expansins [12, 13, 18]. Worth mentioning is also group of non-plant expansins, found in some bacteria [19].

Two main groups of expansins were described: α type, most abundant in dicots [18], assisting in cell separation, wall disassembly and enlargement and β-type, typically found in grass and other gramineous plants [14, 20]. They soften the stigma and stylar tissues, quickening penetration of pollen tubes to the ovule. Even though both types of expansins are only decently connected genetically (about 20% of amino acid sequence similarity [17, 18]), structurally they are homologues with molecules folded into two domains (Figure 5).

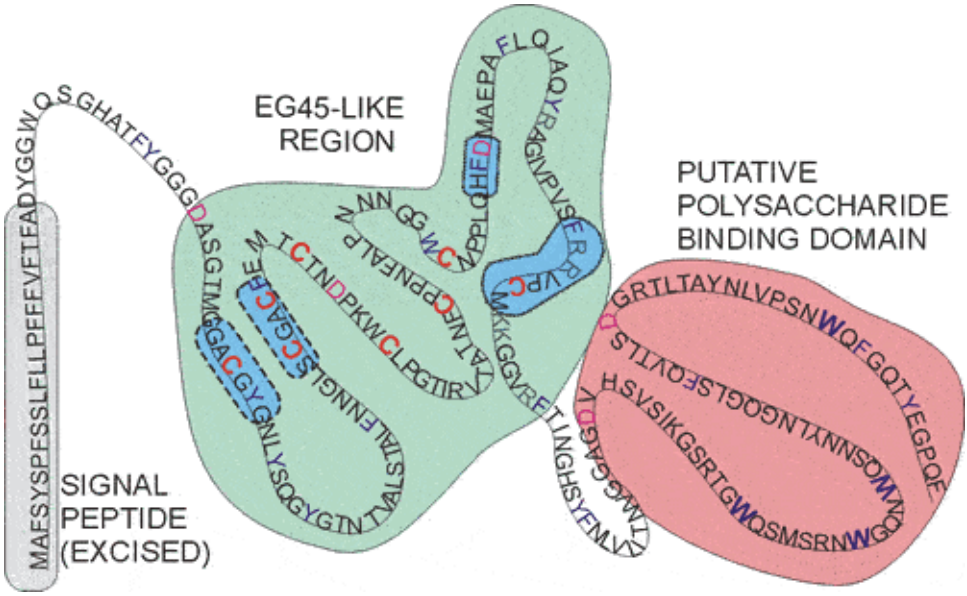

Figure 5. A scheme of expansin protein structure [21]

A typical expansin molecule consists of: a signal peptide, an endogluconase-45 family-like domain and a putative polysaccharide binding domain. The signal peptide is responsible for directing the protein into endoplasmic reticulum/golgi apparatus secretory pathway and is excised when expansin enters into desired location [17].

The first domain is, in terms of amino acid sequence and structure, similar to family 45 of endoglucanases, as it contains His-Phe-Asp motif, typical for enzymes' catalytic site, probably responsible for structure loosening. The second domain is responsible for binding polysaccharides [12, 22].

Expansins are synthesized by numerous plants, including gymnosperms, angiosperms, mosses or ferns. Although many different plants exploit expansins as support for cellulose hydrolysis, their biosynthesis does not differ significantly in different species. It is particularly important that the expansins' sequence is completely different from any known cell wall proteins. Neither it is similar to hydrolases that take part in its degradation, having no motifs in sequence indicating enzymatic function nor to extensins that are cell wall structural proteins, rich in hydroxyproline residues [16].

The exact mechanism of expansins function is still being investigated. They are expected to bind into surface between cellulose microfibrils and other polysaccharides in the cell wall that causes disrupting and weakening of non-covalent bonds [17, 18, 19]. A proposed mode of work of expansin is presented on figure 6.

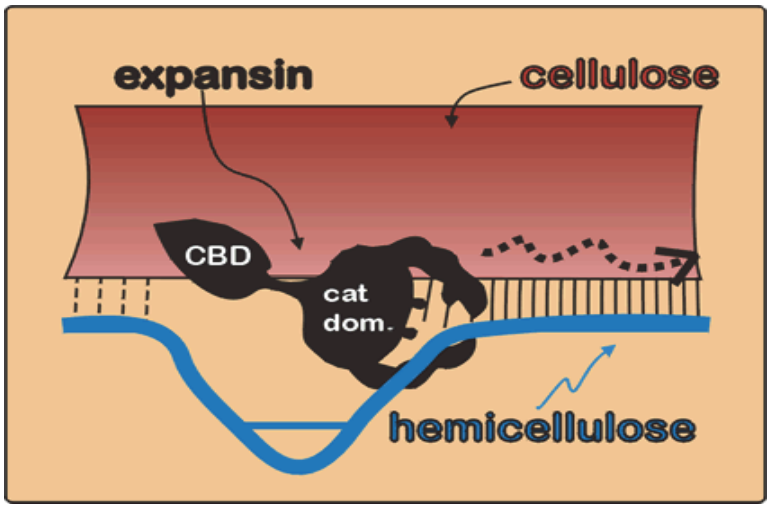

Figure 6. A proposed mode of work of expansin. A catalytic binding domain (CBD) is bound to cellulose microfibrils while the catalytic domain is disrupting non-covalent bonding between cellulose and hemicellulose. By this expanin locally looses the matrix, facilitating enzymatic hydrolysis

Swollenins

This group of proteins are synthesized by fungi. Similarly to expansins, their structure consists of putative signalsequence excised upon secretion, substrate-binding domain and expansin-homology domain [14, 23].

Swollenins have more modular structure and N-terminal cellulose binding module [24], which contain two short sequences that are similar to fibronectin type III, repeats of mammalian titin proteins. The purpose of this feature is unknown, but it is suspected that due to the fact that fibronectin III repeats of titin form β-sheets, enabling protein to unfold and fold. It may make protein albo to stretch thus allow slippage cellulose microfibrils [20, 25].

The biosynthesis of swollenins is induced by certain carbon sources, like sophorose and cellulose. It was found that once aforementioned carbohydrates are present in medium, the expression of swollenin gene was much higher than for another carbon sources [24].

Analogically to expansins their role is to support hydrolysis of cellulose by amorphogenesis weakening structure of the substrate. However, it was found that, unlike expansins, swollenins may possess, albeit very weak, hydrolytic activity [25].

Nevertheless, swollenins cause local disruption of hydrogen bonds between chains of polysaccharides and make short sequences of the chain more accessible for the attack of cellulolytic enzyme. The proposed mechanism of function is shown on figure below:

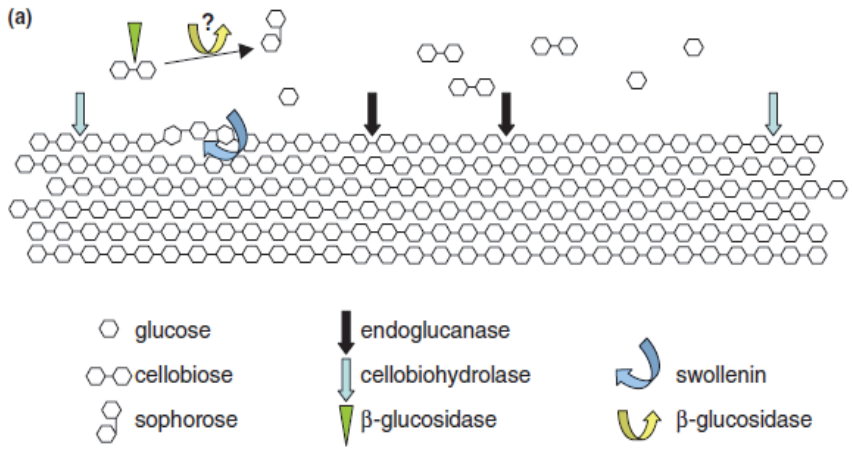

Figure 7. Swollenin disrupting hydrogen bonds, facilitating enzymatic hydrolysis by hydrolases [26]

Loosenin

Loosenin is an another example of protein contributing in hydrolysis of cellulose that voids hydrolytic activity itself. It is synthesized by basidiomycete

Bierkandera adusta [27]. Its structure is different both from expansins and swollenins, lacking putative polysacchirde binding domain, as shown on figure 8.

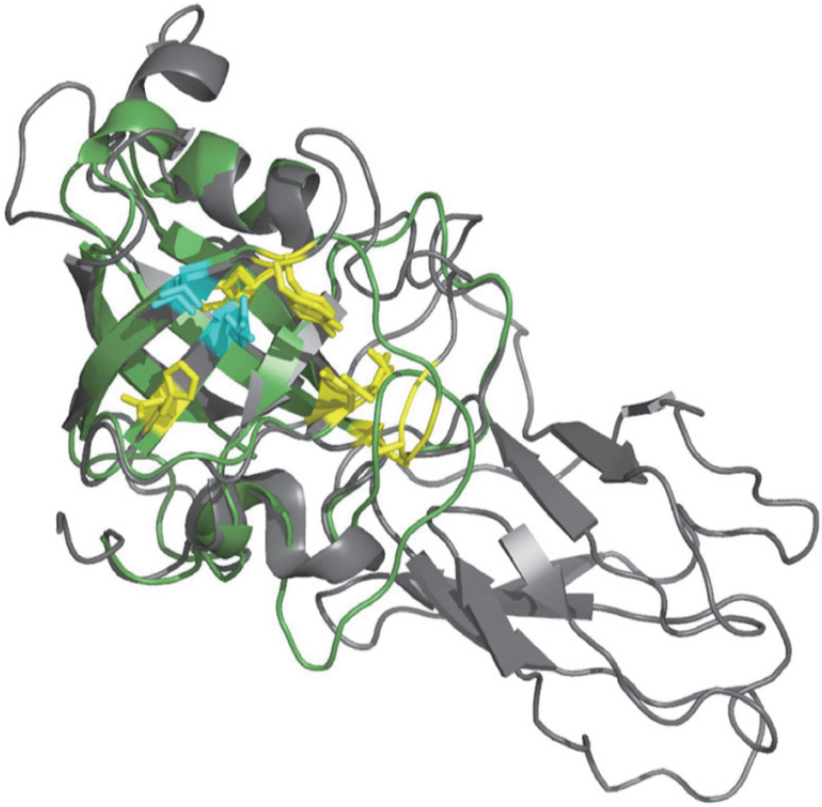

Figure 8. A comparison of loosenin (green) and β-expansin. Due to superposition of both, a decent similarity in spatial arrangement thus secondary and tertiary structure differences are clearly visible [28]

Loosenin mode of workis based on cellulose relaxation is observed as bubbles, promoting amorphogenesis by hydrogen bond disruption, just like swollenin's [24, 28].

Loosenins are able to be expressed it in yeast. Its putative secretion signal is probably recognized by industrially important yeast *Saccharomyces cerevisiae*, as during investigation it was found in culture supernatant [28]. This feature would significantly facilitate production of this protein on industrial scale and its further application in saccharification of cellulose.

Summary

The proteins described in this paper have significant impact on degradation of lignocellulosic complex. Expansins and swollenins are similar in scope of biosynthesis and structure, while loosenin seems to be significantly different.

Expansins, synthesized by plant cells, are considered to bind to the interface between cellulose microfibrils and the other components of lignocellulosic complex, located in the cell wall, and disrupt/weaken non-covalent interactions when in acidic environment. Due to this process, a relaxation of structure occurs, therefore making it much more vulnerable to the enzymatic degradation. Swollenins and loosenins cause local disruption of hydrogen bond between chains of polysaccharides, thus promoting amorphogenesis. It leads to local stress relaxation, making part of the polysaccharide chain prone to hydrolases' attack.

These proteins may be widely applied in the process of depolymerisation in the future, yielding sugar feedstocks for biotechnological production of chemicals and biofuels from the plant biomass. Exploitation of these proteins may at least partially solve problems related to the necessity of pretreatment of lignocellulosic materials prior to their enzymatic depolymerization. Further research into the structure of proteins supporting enzymatic hydrolysis of cellulose, their biosynthesis and genes is necessary. Advances in this field may lead to the construction of their efficient, recombinant producers for large-scale applications.

References

1. Shen Y, Yu S, Ge S. Hydrothermal carbonization of medical wastes and lignocellulosic biomass for solid fuel production from lab-scale to pilot-scale. Energy **2017**, 118:312-323.
2. Zhao X, Liu W, Deng Y, Zhu JY. Low-temperature microbial and direct conversion of lignocellulosic biomass to electricity: Advances and challenges. Renew Sustainable Energy Rev. **2017**, 71:268-282.
3. Morgan HM, Bu Q, Liang J. A review of catalytic microwave pyrolysis of lignocellulosic biomass for value-added fuel and chemicals. Bioresour Technol **2017**, 230:112-121.
4. Nilsson RLK, Holgren M, Madavi B. Adaptability of *Trametes versicolor* to the lignocellulosic inhibitors furfural, HMF, phenol and levulinic acid during ethanol fermentation. Biomass Bioenergy **2016**, 90:95-100.
5. Lee HJ, Lim WS, Lee JW. Improvement of ethanol fermentation from lignocellulosic hydrolysates by the removal of inhibitors. J Ind Eng Chem **2013**, 19:2010-2015.
6. Mattila H, Kuuskeri J, Lundell T. Single-step, single-organism bioethanol production and bioconversion of lignocellulose waste materials by phlebioid fungal species. Bioresour Technol**2017**, 225:254-261.
7. Cai C, Xueqing Q. Using polyvinylpyrrolidone to enhance the enzymatic hydrolysis of lignocelluloses by reducing the cellulase non-productive adsorption on lignin. Bioresour Technol **2017**, 227:74-81.
8. Voet ED, Voet JD, Pratt CW. Fundamentals of Biochemistry. John Wiley & Sons, USA, **1999**, pp. 204-205.
9. Sun Y, Cheng J. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol **2002**, 83:1-11.
10. Gleice G, Denilson A. Obtaining xanthan gum impregnated with cellulose microfibrils derived from sugarcane bagasse. Materials Today: proceedings **2015**, 2:389-398.

11. Xiang Q, Kim JS, Lee YY. A comprehensive kinetic model for dilute-acid hydrolysis of cellulose. Appl Biochem Biotechnol **2003**, 105-108:337-340.
12. Habibi, Y, Lucia LA, Rojas OJ. Cellulose nanocrystals: chemistry, self-assembly, and applications. Chem Rev **2010**, 110:3479-3500.
13. Boerjan W, Ralph J, Baucher M. Lignin biosynthesis. Ann Rev Plant Biol **2003**, 54:519-546.
14. Galbe M, Zacchi G. Pretreatment of lignocellulosic materials for efficient bioethanol production. Adv Biochem Engin/Biotechn **2007**, 108:41-65.
15. Chaturvedi V, Verma P. An overview of key pretreatment processes employed for bioconversion of lignocellulosic biomass into biofuels and value added products. Biotech **2013**, 3:415-431.
16. Cosgrove D, Cell wall loosening by expansins. Plant Physiol **1998**, 118:333-339.
17. Cosgrove D, Loosening of plant cell wall by expansins. Nature **2000**, 407:321-326.
18. Wei W, Yang C, Luo J, Lu C, Wu Y, Yuan S. Synergism between cucumber α-expansin, fungal endoglucanase and pectin lyase. J Plant Physiol **2010**, 167:1204-1210.
19. Bunterngsook B, Eurwilaichitr L. Binding characteristics and synergistic effects of bacterial expansins on cellulosic and hemicellulosic substrates. Bioresour Technol **2015**, 176:129-135.
20. Shan Z, Yang-yang H. The involvement of expansins in response to water stress during leaf development in wheat. J Plant Physiol **2015**, 183:64-74.
21. http://www.personal.psu.edu/fsl/ExpCentral/index.htm
22. Cosgrove D. Plant expansins: diversity and interactions with plant cell walls. Curr Opin Plant Biol **2015**, 25:162-172.
23. Saloheimo M, Paloheimo M. Swollenin, a Trichoderma reesei protein with sequence similarity to the plant expansins, exhibits disruption activity on cellulosic materials. Eur J Biochem **2002**, 267:4202-4211.
24. Andberg M, Penttila M, Saloheimo M. Swollenin from *Trichoderma reesei* exhibits hydrolytic activity against cellulosic substrates with features of both endoglucanases and cellobiohydrolases. Biores Technol **2015**, 181:105-113.
25. Georgelis N, Nikolaidis N, Cosgrove D. Biochemical analysis of expansin-like proteins from microbes. Carbohydr Polym **2015**, 181:105-113.
26. Bocchini-Martins DA, Alves do Prado HF, Leite-Ribeiro RS. Agroindustrial Wastes as Substrates for Microbial Enzymes Production and Source of Sugar for Bioethanol Production. Volume II, Chapter 18. Intech 2011, pp. 319-360.
27. Quiroz-Castaneda R, Anaya-Martinez C, Loosenin, a novel protein with cellulose-disrupting activity from *Bjerkandera adusta*. Microb Cell Factories **2011**, 10:8.
28. Gourlay K, Hu J, Arantes V. Swollenin aids in the amorphogenesis step during the enzymatic hydrolysis of pretreated biomass. Bioresour Technol, **2013**, 142:498-503.