Cellulases: suppliers of energy and basic compounds, so life

Walid Saibi

Biotechnology and Plant Improvement laboratory, Centre of Biotechnology of Sfax (CBS) / University of Sfax, B.P ''1177'' 3018, Sfax –Tunisia

Abstract— *Cellulose is the main constituent of plants, serving to maintain their structure. Indeed, it is a major component of tough cell walls that surround plant cells, and is what makes plant stems, leaves, and branches so strong. Cellulose represents the most abundant carbohydrate substance in nature. It is a bio-polymer of glucose units related by β1, 4 glucosidic linkages. Cellulose degradation requires a multi-enzymatic system composed of three enzymes which are respectively: the endoglucanases, the cellobiohydrolases and finally the β-glucosidases. The chemical industry has come under increasing pressure to make chemical production more eco-friendly and independent to fossil resources. The biocatalysts are the best solution given by nature that can be used to improve some biotechnological applications. The use of this renewable material within the packaging industry has gained increasing interest in the last decades. In this research review, we report some peculiar information's and useful data describing cellulases as biocatalysts, their modulation, implication in a range of metabolic pathways and biotechnological tools.*

Keywords— Cellulose, endoglucanase, exoglucanase, beta glucosidase regulation, catalytic site topology, biotechnological application.

I. INTRODUCTION

Catalysis is a process that increases the speed with which a reaction reaches equilibrium. Since the reaction rate is function of the free energy of activation, in which catalyst causes the decrease of the energy barrier, and thus accelerates the catalytic stage as followed in figure 1-A. Meaning this fact, biocatalysts accelerate biochemical reactions that take place inside or outside of a cell to achieve a speed compatible with its normal operation (figure 1-B).Thus, the operation of a cell's life, reproduction requests that all the reactions occur in a coordinated and controlled but also at a sufficient rate. Enzymes are essential actor's metabolism. Without them, life as we know it would not be possible [1].

The biocatalysts operate on various types of mechanisms that fall into six categories as described in table 1. (1) Acid-base catalysis; (2) Covalent catalysis; (3) Catalysis by metal ion; (4) Electrostatic catalysis; (5) Catalysis by proximity effect and guidance and (6) Catalysis by preferential binding to the transition state complex (table 1). Furthermore, and in addition to their vital character, enzymes have begun in recent decades to be very effective tools in many areas of application such as cosmetic, pharmaceutical, para pharmaceutical, therapeutic, food, etc [1, 2].

Table1

Illustration of the sixth type of catalysis mechanism

Type of Catalysis Mechanisms
Acid-base Catalysis
Covalent Catalysis
Catalysis by metal ion
Electrostatic Catalysis
Catalysis by proximity effect and guidance
Catalysis by preferential binding to the transition state complex

In this research review, we will focus on cellulases that are encoding acid-base catalytic reaction. For this, it is best to start by introducing the natural substrate of this category of biocatalysts which is cellulose.

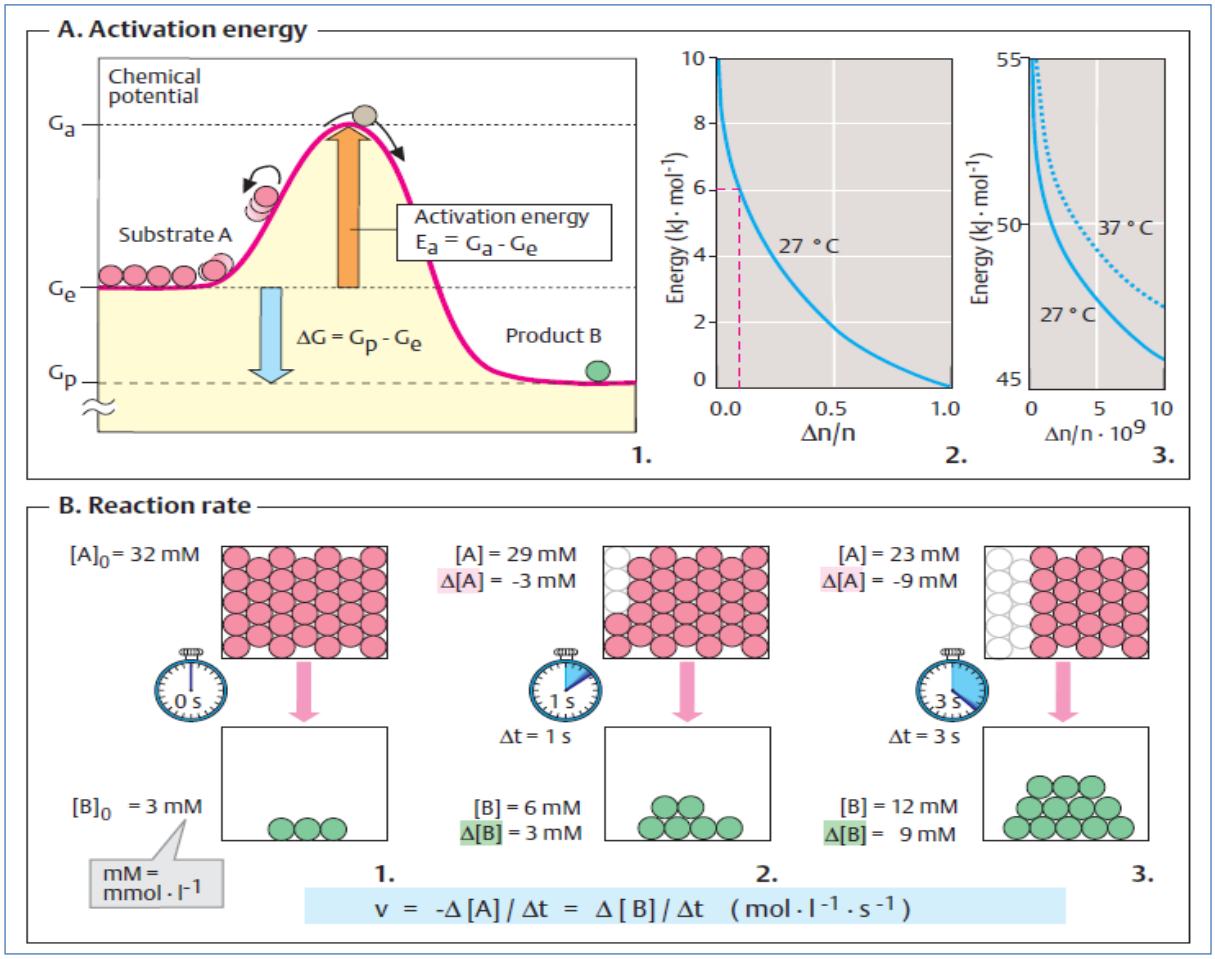

FIGURE 1: A: SHOWS THE EVOLUTION OF THE ACTIVATION ENERGY DURING CATALYSIS PROCESS; B/ILLUSTRATES THE REACTION RATE [6]

II. CELLULOSE: DEFINITION AND STRUCTURE

We note that cellulose is the most common organic substance in the nature and it is estimated that it is half of the total biomass. Its degradation requires a multienzymatic system consisting of three compounds grouped under the denomination of "cellulases' namely endoglucanase, exoglucanase and β-glucosidase [3-5]. Plants have rigid cell walls that allow them to retain their morphology and withstand differences in osmotic pressure between the intracellular and extracellular spaces, which can reach 20 atmospheres. For large plants, such as trees, the cell walls also provide the weight load be a ring function [4]. 10^{15}Kg of cellulose are synthesized and degraded each year. In addition, cellulose is a linear homoglycan consisting of glucose residues linked by β (1,4) bond. Cellulose is a molecule which can reach lengths of 6 to 8 microns [6]. It is mechanically stable and highly resistant to chemical and enzymatic hydrolysis. These properties are due to the conformation of these molecules and their supramolecular organization stabilized by hydrogen bonds (figure 2) [6]. In addition, each channel carries at one of its ends a free aldose moiety having reducing properties allow the assay [7]. The carbohydrate chains are associated on microfibrils via hydrogen bonds and Van der Waals ensuring the cohesion of the fibrils (figure 2) [6]. These chains have crystallin domains separated by amorphous regions. [4].

In the natural state, cellulose fibrils are included in a matrix of hemicelluloses and lignin (figure 2) [6]. The hemicelluloses were composed of a xylan polymer, manann or galactomanann. Often the xylan structure includes various side chains, such as acetyl groups and methyl-arabinofuranosyl, glucuronyl, on which are grafted aromatic compounds (ferulic acid, para-coumaric acid).The latter are involved in the coupling of the hemicelluloses from the lignin via ether bridges (figure 2) [6]. Cellulose is also a potentially important source of solvents and fuels (acetone, butanol, ethanol, acetic acid etc.) that may be

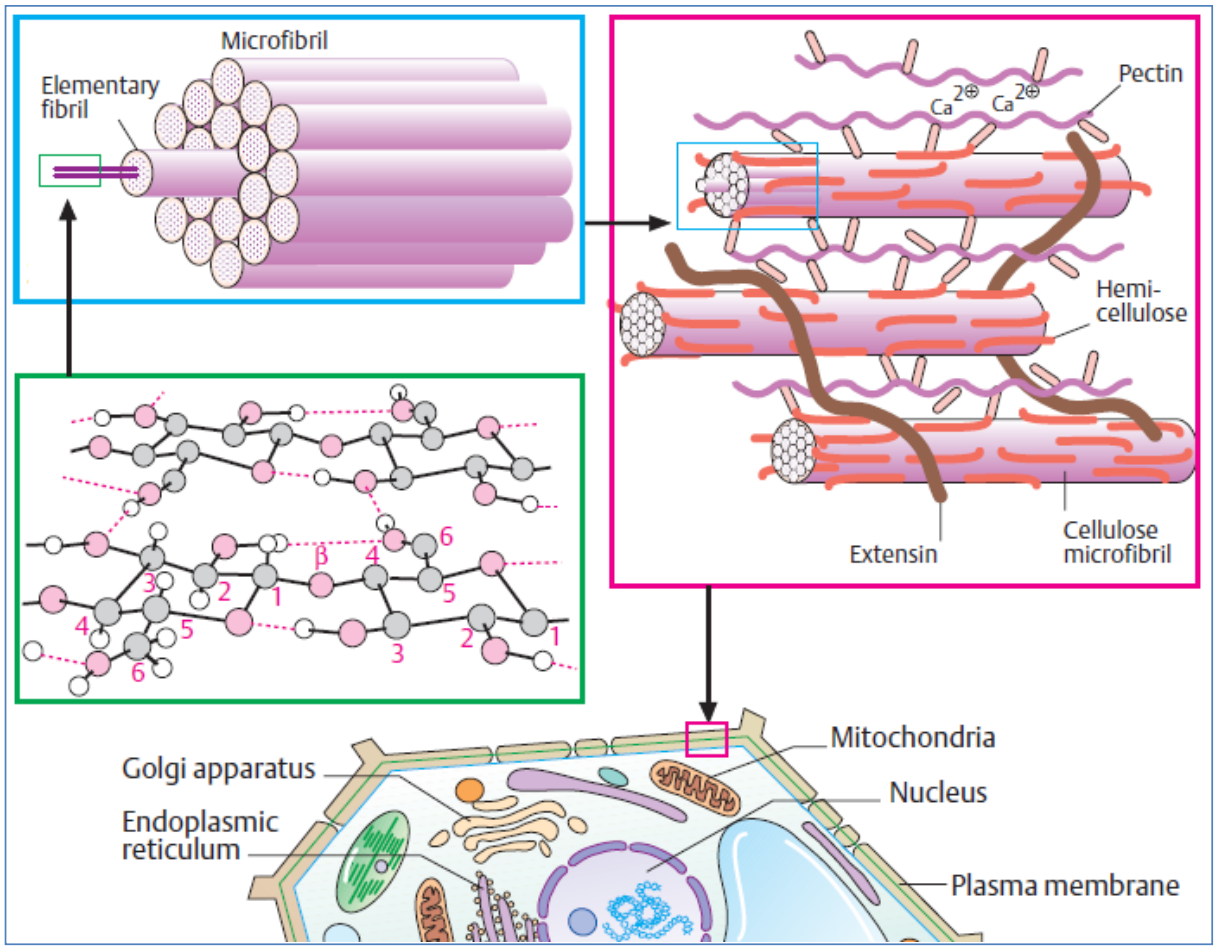

FIGURE 2: CELLULOSE STRUCTURE, CONFORMATIONAL ORGANIZATION AND HIS INVOLVEMENT ON THE CELL WALL [6]

obtained by fermentation from glucose resulting from hydrolysis thereof [8, 9]. Those chemical compounds are requested in various fields as good alternatives for some of basic needs for life, especially in the pharmaceutical and clinical fields

III. CELLULASES

In the wild and up to the date of writing these lines, there is no enzyme able alone to degrade cellulose. His depolymerization involves quite a heterogeneous enzyme system consists of three types of cellulolytic acting synergistically. These glycosyl hydrolases catalyze the hydrolysis of glycosidic linkages in polysaccharides, oligosaccharides and their combinations based on glucose and cellobiose units [10]. Cellulases are classified as families of the "Glycosyl Hydrolases" whose number is currently about 133 families (http://www.cazy.org/Glycoside-Hydrolases.html).

Under the cellulases generic term refers to three activities: endoglucanases (EC3.2.1.91) exoglucanases (EC3.2.1.4) and β-glucosidase (EC3.2.1.21). However, a fourth enzyme named cellobiose dehydrogenase or CDH (EC 1.1.99.18), could be considered as part of this system by sharing with β-glucosidase the same natural and so physiological substrate (cellobiose) [1, 3]. This fact is clearly proved in figure 3. These cellulolytic enzymes were purified and studied from several origins and their biochemical properties have been well studied due to their activity on specific substrates (natural, synthetic, chromogenic and fluorogenic ones) [11-13].The vast heterogeneity of cellulase preparation and the multiplicity of enzymes for the same activity, as is the case for most of the "carbohydrate hydrolases" make quite complex splitting of the various components [1, 5].

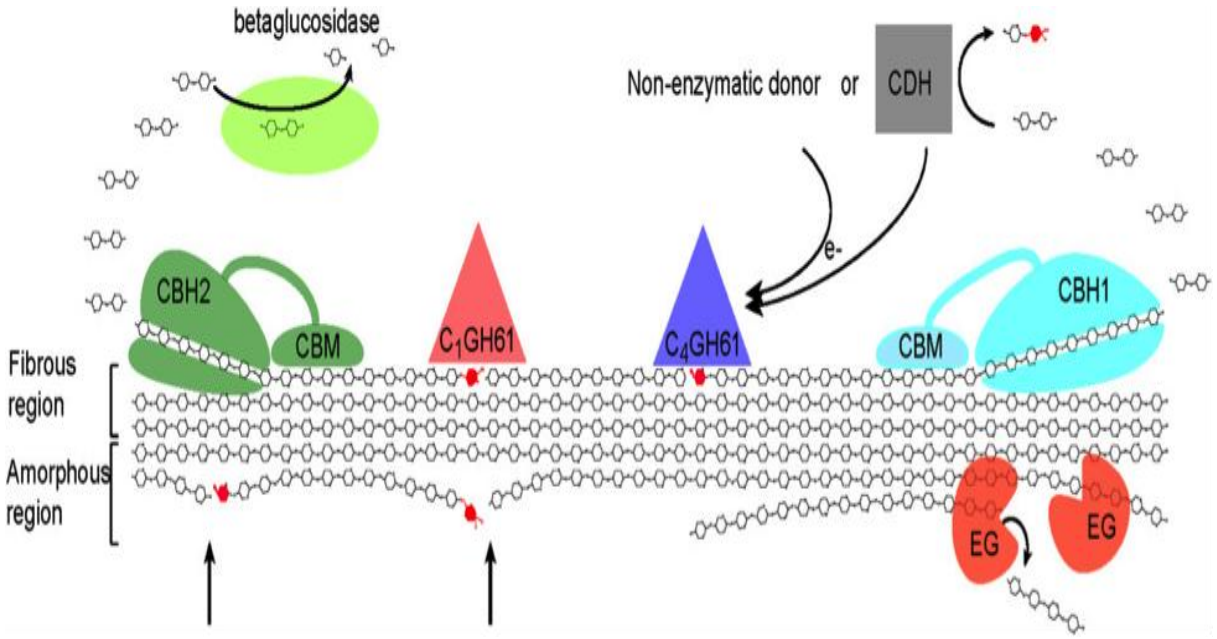

FIGURE 3: PLOT RECAPITALIZING THE MODE OF ACTION OF CELLULASE SYSTEM, PLAUSIBLE IMPLICATION OF THE CDH IN CELLULOSE DEGRADATION PROCESS AND ALSO DESCRIBING THE TWO TYPES OF CBH (CBH1 AND CBH2).THIS FIGURE WAS COLLECT FROM

(https://www.google.fr/search?tbm=isch&q=cellulose&hl=fr&authuser=0)

IV. ENDOGLUCANASES

These biocatalysts hydrolyze glycosidic linkages of β- (1,4) by driving unordered cuts in the amorphous regions of the cellulose chain and more particularly on the highly substituted cellulose such as carboxymethylcellulose (CMC) and hydroxyethylcellulose (HEC) [14]. The action of these enzymes on such substrates is led to a release of reducing sugars and a decrease in the viscosity of the substrate. They are active on oligosaccharide chains but it has no effect on cellobiose [14].

The endoglucanase action on the cellulose-crystalline regions is much reduced or absent for most of them. The speed with which they hydrolyze the substrate is function of the length of the polysaccharide chain [15, 16]. We note that in the same cellulase preparation, protein species with endoglucanase activity out number cellobiohydrolases. The endoglucanases act generally at acidic pH and very few of them act at basic pH, such as those from Humicola insolens and recently discovered in *Stachybotris microspora* [14].

In contrast, there are many cases of bacterial basic endoglucanases such as endoglucanase 1 from Streptomyces sp and endoglucanases EH and EL from Bacillus sp whose optimum pH is 8.5. In addition, an alkaline endoglucanase were characterized in the green beans and another has been described only in terms of the abscission zone of the plant tissue after a stress induced by ethylene [17, 18].

We note also that the results of various recent studies corroborate, and expand on, previous experiments identifying pathways of cell-wall synthesis and breakdown as being induced during abscission in immature fruits, flowers, and leaves [18-20]. Indeed, great numbers of cell-wall-related genes were up-regulated in fruit-abscissic zone, such as some of carbohydrate enzymes may be required for complete cell separation in mature fruit, and possibly for wall restructuring after abscissic zone-cell separation. These genes encode for abscission-associated cell-wall hydrolyzing enzymes like endoglucanases [21].

Recent characterization of several cell wall mutants has revealed a causal link between cell wall synthesis and structure and the ethylene signalling pathways. These mutants were originally isolated from screens for ectopic lignin production and defense responses. Indeed, they revealed that the mutations responsible for these phenotypes lie within different highly conserved regions of the *cellulose synthase* genes. In addition, in plants which are mutated in an endoglucanase, it appears

that the induction of the defense-related genes is responsible for the ectopic production of lignin in cellulose-deficient plants [22].

These biocatalysts are very required in some industrial applications due to their alkalinity [14]. They not only differ from each other in the composition of the hydrolysis products, but also according to their absorbability to the substrate. Indeed, the *Tricoderma. reesei* endoglucanase III is small which lacks cellulose binding module. As a consequence, it cannot be adsorbed to the substrate. These enzymes are also distinguished by their synergistic interaction with cellobiohydrolases. The endoglucanases were classified into two categories: (1) ENDOGLUCANASES "ENDO LIKE" that they attack preferentially the glycosidic chain, resulting in the rapid reduction of the viscosity of the CMC, rather than the appearance of the reducing sugars is very slow; and (2) Endoglucanases «Exo like» witch attack preferentially the chain at its end encoding the release of reducing sugars faster than the reduction of the viscosity of the substrate [1]. The analysis of the degradation products of these enzymes and the use of chemically abeled oligosaccharides was used to distinguish between these two types.

Structurally, endoglucanases are formed of three domains: a catalytic domain, a binding domain for cellulose and a third unit linking the two first ones. The latter has a very flexible structure that allows the enzyme to adopt a three-dimensional conformation more suitable for a better catalytic efficiency during the catalytic process [1].

The endoglucanase catalytic site is characterized by an acid catalytic dyad type, which performs its function through acid-base catalysis [1, 9]. Indeed, one of the two catalytic amino acids represents a nucleophilic center and the other one is a protonated center. The binding domain to cellulose is known under the name of CBM for "Cellulose Binding Module" [1]. Determining the amino acid sequence of more than one hundred of fungal CBM showed the strong conservation of a sequence of amino acids and appears to be the most conserved domain in nature. In fungi, the CBM has a size of about 33 to 44 amino acids. It is 98 to 108 residues in bacteria [23, 24]. Based on their properties, CBMs are grouped into 71 families that display substantial variation in substrate specificity, along with other properties that make them a gold mine for biotechnologists for diverse and unusual applications (http://www.cazy.org/Carbohydrate-Binding-Modules.html).

In recent years the practical use of CBMs has been established in different fields of biotechnology. Three basic properties have contributed to CBMs being perfect candidates for many applications: The first one results in the fact that CBMs are usually independently folding units and therefore can function autonomously in chimeric proteins; The second one is related to the attachment matrices that are abundant and inexpensive and have excellent chemical and physical properties; and the third one concerned the binding specificities that can be controlled, and therefore the right solution can be adapted to an existing problem. The major application for CBMs, given that large-scale recovery and purification of biologically active molecules continue to be challenges for many biotechnological tools [25]. The second major use of CBMs results on cell immobilization technology that ranges from ethanol production and phenol degradation to mammalian cell attachment and whole-cell diagnostics [25-27].

V. CELLOBIOHYDROLASES

Cellobiohydrolases or exoglucanases form the second component of the cellulase system. They act on reducing and non-reducing ends of cellulose chains or cellodextrins, at crystalline regions. These enzymes are called processive cellulases which slide along the chain, releasing sequentially and mainly cellobiose. They have no effect on the substituted cellulose (CMC and HEC). We report the presence of two classes of cellobiohydrolases: (1) Cellobiohydrolases type 1 or CBH I: They attack the cellulose at its reducing end. They represent the most significant portion in the cellulolytic complex (40 to 60% of total protein) and (2) Cellobiohydrolases type II or CBH II: They attack the cellulose at its non-reducing side and release cellobiose as for CBHI [1].

Exoglucanases, as endoglucanases, have a structure consisting of three fields: a CBM, a catalytic domain and an anchoring bridge or "linker" that joins the first two ones. We also note that CBHI and CBHII of T.reesei differ from the location of the cellulose binding domain, as it is on the C-terminal side in the first and on the N-terminal side in the second as followed in figure 3.

VI. CATALYTIC SYNERGISM BETWEEN THE ENDOGLUCANASES AND THE EXOGLUCANASES

Figure 4 referring catalytic acts of endoglucanases and cellobiohydrolases and synergism that could exist between these two cellulolytic components upon hydrolysis of cellulose. Catalytic act leading to the total hydrolysis of the organic polymer is completed by the action of the third component of the cellulase system which is the β-glucosidase, ensuring conversion of cellobiose and small oligosaccharides into glucose units, easily metabolizable during cell growth and convertible energy,

usable in biotechnology fields [28]. If we considered that CDH can be added to the cellulase system compounds, figure 3 illustrates the contribution of CDH in the cellulose degradation process via the conversion of the cellobiose into cellobionolactone as described in [28].

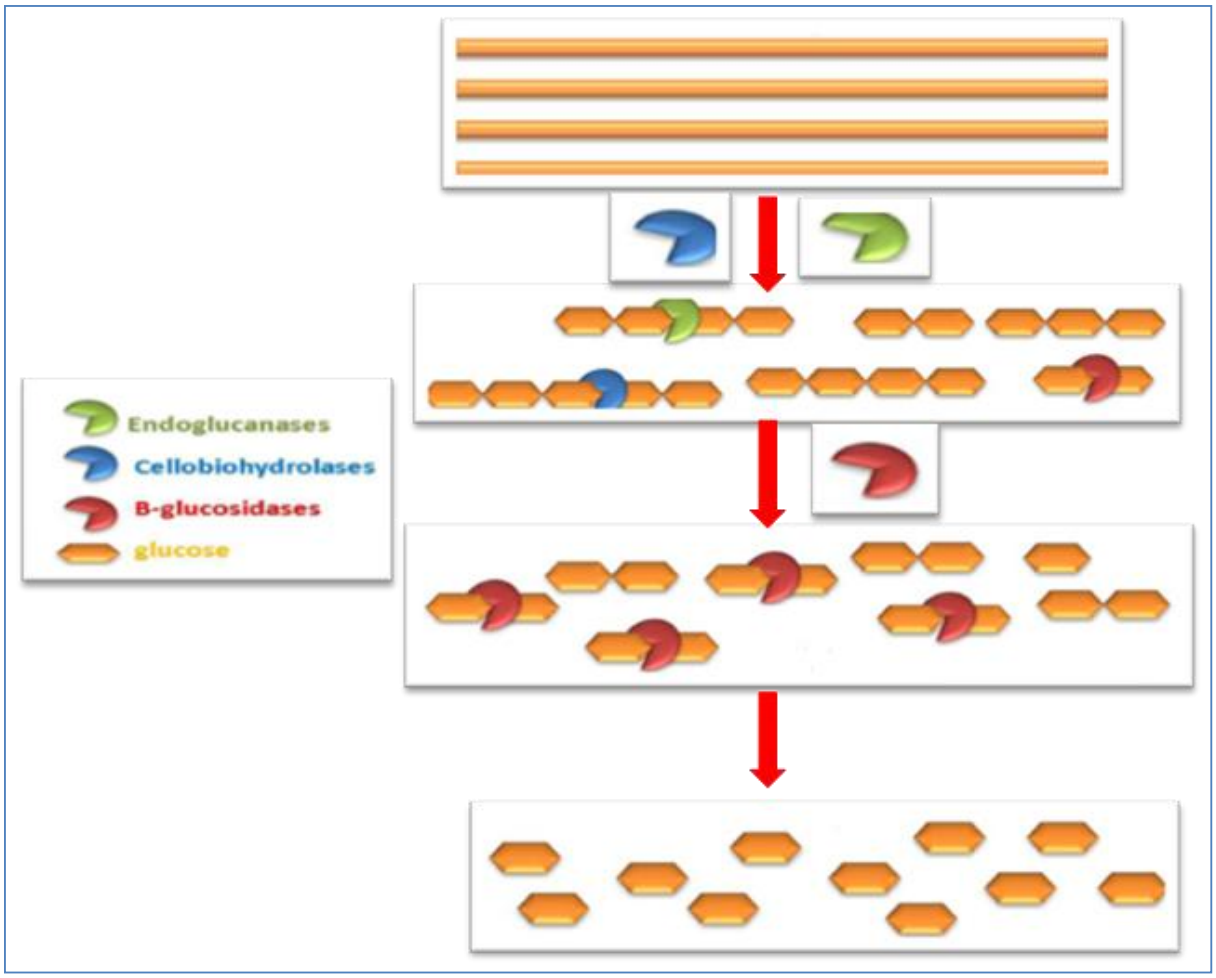

FIGURE 4: MODE OF ACTION OF CELLULASIC SYSTEM COMPOUNDS

VII. *B*-GLUCOSIDASES

The β-glucosidase or cellobiase (EC3.2.1.21) catalyzes the hydrolysis of cellobiose as well as aryl and alkyl glucosides and small oligosaccharides containing β1-4 linkages [13]. The β-glucosidases are ubiquitous enzymes, unlike other components of the cellulase system. Indeed, they are present in the majority of living beings (animals, plants, insects, bacteria, fungi) [28, 29].

These enzymes, whatever their origins, show a remarkable similarity concerning the specificity for natural and some of non-physiological substrates. We note, however, that they can be a big difference for the aglycone specificity of the physiological substrates (aryl, alkyl) [9, 12, 13]. They are an important group of families of the "Glycosyl Hydrolases" (GH) which is the subject of several recent researches with the potential roles that could be played by this enzyme class basically in biotechnological fields [11, 12].

7.1 Mode of action of cellulasic system compounds

As we noted as showen in figure 4, the biodepolymerisation of cellulose involves endoglucanases, exoglucanases and β-glucosidases. This hydrolytic and synergistic action begins with the endoglucanase, random cuts through to the interior of the polymeric structure within the amorphous regions, releasing glucose, cellobiose and oligosaccharides and thus reducing and non-reducing ends. These serve as anchor for exoglucanases which act with processive mode liberating cellobiose, β-

glucosidase substrate that accomplishes this cellulolysis to generate glucose: carbon source of useful energy and metabolites for the microorganism and cells in general.

Intermediate and final reaction products are inhibitors of endoglucanases and especially exoglucanases. The elimination of this inhibition is through the β-glucosidase [29]. Thus the action of this biocatalyst is judged essential for the removal of this inhibition during the hydrolytic process.

7.2 Topology of cellulase catalytic sites

The enzymatic activity is dictated by the presence within its structure (secondary and tertiary), a so-called active site [30, 31]. Schematically, it has the shape of a cavity or a groove into which will bind the substrate through ionic bonds. Once attached, the substrate is being turned into product. The active site is subdivided into two parts: the binding site / binding / recognition (which interacts with a form fit with the specific substrate to the enzyme) and the catalytic site (which allows the flow of the substrate conversion reaction product) [31]. The majority of classes of enzymes have representatives whose three-dimensional structure was established. The catalytic site of cellulases is composed of a catalytic diad acid type, formed by two aspartic acids, glutamic acids, or both aspartic and glutamic acid [32]. Analysis of the cellulase's structure suggests that their catalytic sites shaped pocket, groove or tunnel topology as showed in figure 5 [33]: The first one (fig 5-A) is recovered in the enzyme catalytic sites of monosaccharidase activity such as β-glucosidase; The second one (fig 5-B) is characteristic of enzyme with a mode of action "Endo" where a cellulose chain can be internalized into the groove and be cleaved and the third one (fig 5-C) is met with enzymes having a mode of action such that said processive cellobiohydrolases. This topology is similar to that site groove shape; clean the endoglucanases, except that two loops close the furrow to create a kind of tunnel. These enzymes will remain attached to the cellulose chain and progressing along the latter and releasing mainly cellobiose [33].

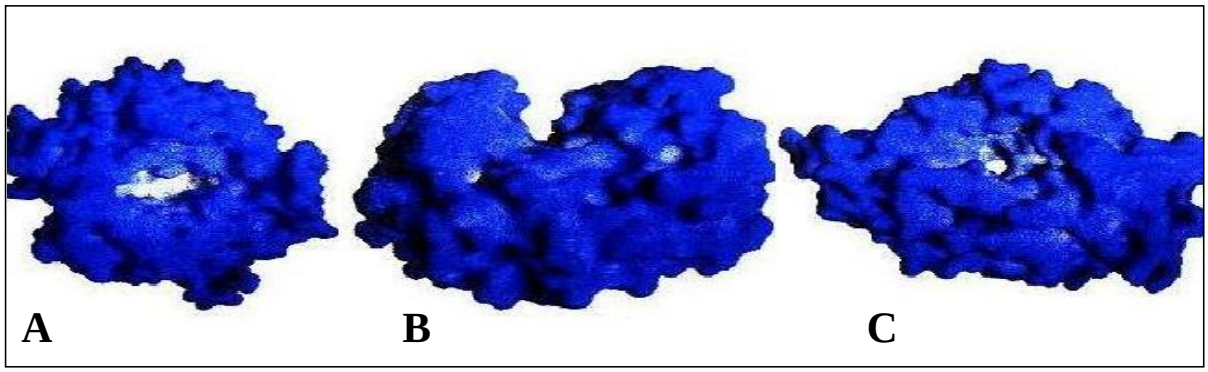

FIGURE 5: ILLUSTRATION THE TOPOLOGY OF THE CATALYTIC SITE IN WHICH A: THE ENZYME CATALYTIC SITES OF MONOSACCHARIDASES SUCH AS B-GLUCOSIDASE; B: THIS TOPOLOGY IS CHARACTERISTIC OF ENZYME WITH A MODE OF ACTION "ENDO" WHERE A CELLULOSE CHAIN CAN BE INTERNALIZED INTO THE GROOVE AND BE CLEAVED AND C: THE TOPOLOGY IS MET WITH ENZYMES HAVING A MODE OF ACTION SUCH THAT SAID PROCESSIVE CELLOBIOHYDROLASES AS ILLUSTRATED BY [33]

7.3 Production regulation of cellulases

Protein synthesis by living organisms is subjected to regulation mechanisms. Although cellulose is a biopolymer of relatively simple structural, compared with most other natural polymers (hemicellulose and pectin), it requires for its degradation enzyme complex that is most often inducible in fungi and this induction is function of several parameters such as the carbon source, the nitrogen source, growth temperature, ventilation, light, medium pH, granulation size, the type of fermentation, etc.... [5, 14, 34]. With molecular biology techniques that are constantly changing, the transcripts of a number of genes encoding cellulases have been characterized in different organisms. Studies show that these genes are monocistronic and the regulation of their expression is mainly at the transcriptional level [9, 35]. Physiological, biochemical characterization and quantification of the mRNA of the genes have shown that the cellulases are inducible. However, the interpretation is often complicated by the absence of free inductor, hence the interference between the substrate and induction by its own catabolism.

On the other hand, it is clear that the synthesis of cellulase is subjected to catabolic repression in the presence of low molecular weight substrates and more easily metabolized as cellulose [29, 36]. Nevertheless, there is for many systems, suggestions that strongly support the idea that this production is not regulated only by the absence and presence of the carbon source but also a quantitative threshold that induces or represses this induction. Since cellulose is a water-insoluble substrate and as it is unable to cross the cell membrane and induce the cellulase system; it is generally accepted that the constitutive basal level cellulases frees disaccharides and soluble cellooligosaccharides with the ability to cross cellular biological barriers and induce cellulase system for effective induction for good growth and a good work cell.

7.4 Saccharides: potential inductor's of cellulases

Various cellooligosaccharides and derivatives thereof are used to identify the cellulase inductors. Cellobiose, smaller cello-oligosaccharide with β1-4 linkage, is reported as inducing some cellulases. Sophorose (disaccharide of glucose with a β1-2 bond) induces cellulases from *Humicola jecorina* with a factor of 2500 folds larger than cellobiose [37]. Studies in regulation of cellulase system of Trichoderma reesei (considered as E. coli in eukaryotic model in the field of cellulases) have shown that theirβ-glucosidase is co-induced by sophorose, with endoglucanases and exoglucanases [38]. In fact, they are actually induced, not by what disaccharide but rather by the Methyl-β-D-glucoside that against this by no effect on the induction of other cellulase activities: this suggests that (i) the β- glucosidase is not subject to the same regulatory system as the other components of the cellulasesystem of Trichoderma reesei; (ii) there is a metabolic pathway or an enzymatic activity that is responsible for the production of this type of inductor [38].

7.5 Inhibition of cellulases

By definition, an inhibitor is a substance that decreases the rate of the reaction catalyzed by an enzyme. By binding to biocatalyst, inhibitor can prevent the substrate binding at the active site [39, 40]. The inhibition of the enzyme plays an important role in controlling the biological mechanisms and in particular in the regulation of metabolic pathways. In enzymological studies, inhibitors are often used to determine the mechanism of action of an enzyme. Applications exist in many other areas, more drugs, pesticides and insecticides are enzyme inhibitors [41].

Seek for new glycosidase inhibitors has fundamental and applied interests, because glycosidases are involved in many metabolic pathways. Failure in these enzymes causesa range of metabolic. Selective glycosidase inhibitors may have potential applications in order to alleviate and / or cure the sick. They can also be used also as probes to study the topology of the active site of these enzymes and thus to understand the relation "structure-function" of such enzyme. The affinity of an inhibitor for the enzyme is given by the inhibition constant Ki, which is the inhibitor concentration at which half of the enzyme site is occupied. Thus, the affinity of an inhibitor is even greater than the Ki is small [42].

7.6 Action mode of β-glucosidases

Like other cellulases (endoglucanases and exoglucanases), β-glucosidases exert their catalytic act through a catalytic diad acid type, in which the first one is a nucleophile and the other is in the protonated state. Enzymatic catalysis of the β-glucosidase and monosaccharidases, in general, is provided with a catalytic structure in the form of a "pocket" or "crack" with accommodation and hydrolyzing disaccharides, oligosaccharides and some other structural analogs. Indeed, β-glucosidases are grouped into three groups: The first group hydrolysis aryl glycosides;the second group which hydrolyzes only cellooligosaccharides whose cellobiose and the third one cleaves both aryl as cellobiose and oligosaccharides [1, 11, 12]. The catalysis takes place in two different mechanisms; one preserves the configuration "β" of the anomeric carbon, and the other inverts "β" to "α".

The first one named "Inversion of the configuration"**,** in which The nucleophile is located in a relatively remote area of the anomeric carbon. The oxygen in the glucoside bond is protonated by the acid-base residue; an activated water molecule binds to the glycosyl residue with inversion of the stereochemistry of the anomeric carbon. The distance between the two catalytic residues is about 9.5 Å, which allows having the space to accommodate a water molecule at the same time as the substrate. Thus, the catalytic act takes place in a single step by co-hosting of the molecule of the substrate with a water molecule and thus enables the conversion in a single step with a single shift function and the enzyme is thus called type "inverting enzyme".

In the second type called "Retention of the configuration**",** the nucleophile is closer to the anomeric carbon. The distance between the two catalytic residues is about 5.5Å. The oxygen in the glucoside bond is protonated by the acid-base residue; the nucleophile is directly involved in the departure of the aglycone. This results in a covalent glycosyl-enzyme intermediate

with a first inversion of the stereochemistry of the anomeric carbon (from β to α form). The glycosyl-enzyme is hydrolyzed in a second step involving a water molecule which causes a second inversion of the stereochemistry of the anomeric carbon (from α to β form). Then the reaction precedes in a double function of displacement, hence the name "Retaining enzyme".

7.7 The β-glucosidase: ubiquitous enzyme

β-Glucosidase is an ubiquitous enzyme and can be found in bacteria, fungi, plants and animals. It has enormous biotechnological applications. Its deficiency in natural enzyme preparations is often overcome by exogenous supplementation, which further increases the enzyme utilization cost [43, 44].

7.8 The β-glucosidase from insects

The β-glucosidase in insects shares some properties with those of plants. Linamarase of Zygae natrifolii is a glycoprotein responsible for the hydrolysis of some cyanogenic substrates at the hemolymph. In vitro, it is inhibited by Mg2+ ions and Cu2+ to an uncompetitive manner and sometimes mixed. Recently a very interesting result was reported: there is some homology between β-glucosidase and delta endotoxins of B. thuringiensis used as bio-insecticide [45, 46]. Indeed, the anti-delta endotoxin antibody recognizes epitopes of β-glucosidase and the opposite is true. In the same context, the β-glucosidases are endowed with insecticidal capacity against not only insect larvae, as is the case of the B.thuringiensis delta-endotoxins, but also against the adult stage of the insect [45, 46].

7.9 Bacterial β-glucosidase

The bacteria also possess β-glucosidases, among which we can mention the trans-glycosilases lytic enzymes that are involved in the maintenance and growth of bacterial peptidoglycan cell wall [47]. They cut β1-4-glycosidic bonds of its kind in the peptidoglycan forming non-reducing anhydro-muropeptidoglycanes. We note also the presence in E. coli operons encoding cryptic phospho-β-glucosidases, which may be activated upon insertion of sequences in their promoters [47, 48].

7.10 The phyto-β-glucosidases

They have been known since 1837 by Liebigand Wohler [36]. In plants, the β-glucosidases are involved in several metabolic events as well as growth [49]. They are active in the defense against pathogens and herbivores by the release of para-coumaric acid, thiocyanic, terpeneand cyanides; or by hydrolyzing the conjugates phytohormones (glucoside gibberellins, auxins, abscisic acid and cytokinins) [49-51]. However, most of the functions postulated forphyto-β-glucosidases are not yet well documented and clarified and make a great field of investigation. The primary structure of genes encoding phyto-β-glucosidase is known, as for example in the clover. Although plants do not have a real immune system directed against external aggression, they have developed defenses against herbivores based on the release of toxins from cyanogenic glycosides stored by the plant [50, 51]. This reaction is performed through some of the β-glucosidases baptized: cyianogenic β-glucosidases that with their glucoside substrates stored in multiple tissue compartments [52]. Indeed, as a result of damage caused by stress (cold, insects...), there is the release of endogenous β-glucosidase and hydrolysis of toxic substrates. We then witness the release of conjugated bitter toxic aglycones as well as their degradation products such as thiocyanate, isothyocyanates, nitryles, alkaloids, terpenoids, saposin, benzaldehyde, hydrocyanic acid (HCN) [50, 52]. In another hand, the phytohormones are synthesized as pro-hormones, inactive due to their binding to aglycone compound. If necessary, the β-glucosidases release hormones in their active form, such as auxins, hydrolyzing the glycosidic bond. These glycosidic substrates, phytohormones precursors, are localized in the endosperm and are transported during germination to other parts of the seed. In addition, we note that β-glucosidases play a crucial role in the synthesis of lignin. In fact, various authors haves shown the involvement of the β-glucosidase localized at the wall in the synthesis of lignin through the release of certain precursors of lignin [53].

VIII. SOME EXAMPLES OF APPLICATIONS OF CELLULASE SYSTEM

The production of fuels and chemical compounds from lignocellulosic biomass is the most known application using cellulase capacities. Concerning this fact, the heterogeneity and recalcitrance of plant cell walls in a cost-effective manner at scales sufficient to offset fossil-fuel-derived resources is a major technical challenge. The production facilities of the biofuels second-generation are now days under development, with a primary purpose to convert lignocellulosic biomass to ethanol. These facilities generally utilize a “biochemical conversion” process wherein biomass is first size reduced through milling or chipping, followed by a mild physico-chemical pretreatment step to render plant cell wall materials more amenable to attack by biological processes. The process is summarized in figure 6 [9].

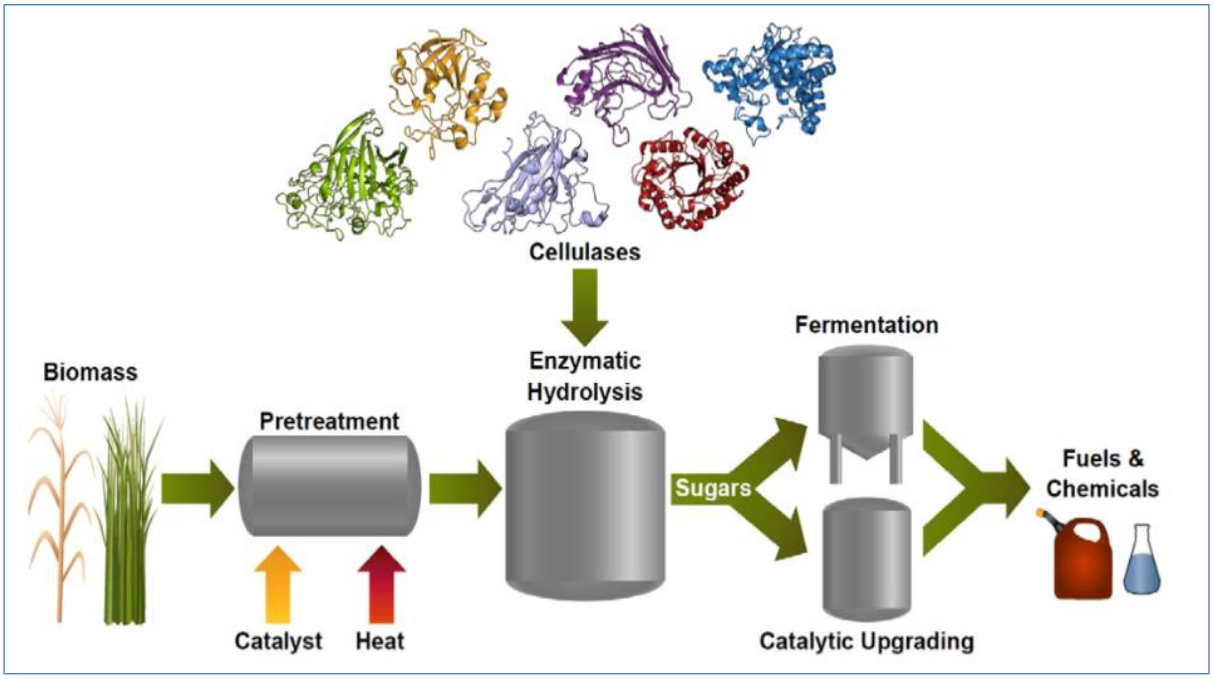

FIGURE 6: OVERALL VIEW OF THE PLAUSIBLE BIOCHEMICAL CONVERSION PROCESS TO PRODUCE FUELS AND CHEMICAL COMPOUNDS FROM LIGNOCELLULOSIC BIOMASS, USING CELLULOSIC SYSTEM [9]

In another hand, there are other applications of cellulases such as their use as alternative in Yeast DNA Extraction. Indeed, *Stachybotrys microspora* is a filamentous fungus characterized by the secretion of multiple hydrolytic activities. The production of these biocatalysts was studied under submerged culture. Endoglucanases, pectinases, xylanases, β-glucanases, chitinases, and proteases were induced on cellulose-based medium and repressed on glucose. The yield of chitinases, β-glucanases, and proteases produced by *Stachybotrys* strains was as much higher than the commercialized lysing enzyme called "zymolyase," currently used in yeast DNA extraction [54].

1) The β-glucosidase is one of the compounds of the cellulase system. It was used on the conversion of oleuropein into Glucose and Hydroxytyrosol. Indeed, the olive tree has been accepted as a symbol of holiness, abundance, wisdom and health, for centuries. Although its fruit is mainly used as a food after processing for table olive and olive oil, it is used for preparing medical products, cosmetics and animal feed. There are many researches that have been done on the effects of oleuropein, one of the most important phenolic compounds extracted from olive leaves, on health. BglG, a fungal β-glucosidase [55], possesses the capacity to cleave oleuropein with a recovery of 88 % after 24 h of reaction. This result gives birth to the plausibility of bglG to be used as a potential biological tool in agro-food domains such as eliminating the bitter taste of olives [10].

Enzymes are also used in silage to degrade biopolymers into simple sugars contained in the cells and in cell walls. Theoretically enzymes indirectly increase the content of soluble sugars in the forage for use by lactic acid bacteria. If it's possible increasing fast enough concentration of soluble sugars, it should follow a more marked lowering of the pH and a better preservation of the silage. Two categories of enzymes can be distinguished according to their role. The first one attacks the cell contents (eg amylase, pectinase) to provide more sugars for fermentation. As against the second one represented mainly by the cellulases attacks the cell walls and influences the silage quality in two levels. (1) More releasing soluble sugars and (2) they should increase digestibility forages by reducing the fiber content. [56].

IX. CONCLUDING REMARKS

Cellulases have evolved to be the most powerful and prevalent biomass degrading system in nature, exhibiting the turnover of lignocellulosic material on Earth. Given their significant activity and ability to be readily produced at high titers on the industrial scale, cellulases are considered as an excellent solution and strategy for biotechnological applications.

In another hand, cellulases, some of which remain to be discovered, work in concert to accomplish one of the most important processes in nature, namely the turnover of cellulose. This process is of paramount importance in the global carbon cycle and may become one of the most biotechnological important enzymatic reactions given the desperately needed drive toward a renewable energy-based global society.

As reported in this case, significant strides in our fundamental understanding of cellulase action have been made in the past several decades, especially driven by the structural biology efforts starting 20 years ago. However, we will strive to enhance and ameliorate the level of exploration of these natural capacities in order to improve the Human life.

ACKNOWLEDGEMENTS

This work was supported by grants from the Ministry of Higher Education and Scientific Research of Tunisia.

REFERENCES

[1] Saibi, W., et al., Biocatalysts: Beautiful creatures. Biochemical and Biophysical Research Communications, 2012. 426(3): p. 289-293.

[2] Saibi, W., et al., Empiric, structural and in silico findings give birth to plausible explanations for the multifunctionality of the wheat dehydrin (DHN-5). Acta Physiologiae Plantarum, 2015. 37(3).

[3] Saibi, W. and A. Gargouri, Cellobiose dehydrogenase influences the production of S. microspora β-glucosidase. World Journal of Microbiology and Biotechnology, 2012. 28(1): p. 23-29.

[4] Saibi, W., B. Amouri, and A. Gargouri, Purification and biochemical characterization of a transglucosilating β-glucosidase of Stachybotrys strain. Applied Microbiology and Biotechnology, 2007. 77(2): p. 293-300.

[5] Saibi, W., S. Abdeljalil, and A. Gargouri, Carbon source directs the differential expression of β-glucosidases in Stachybotrys microspora. World Journal of Microbiology and Biotechnology, 2011. 27(8): p. 1765-1774.

[6] Koolman, J. and K.-H. Röhm, Color atlas of biochemistry. 2005: Thieme.

[7] Abdeljalil, S., et al., Improvement of cellulase and xylanase production by solid-state fermentation of Stachybotrys microspora. Biotechnology and Applied Biochemistry, 2014. 61(4): p. 432-440.

[8] Chundawat, S.P., et al., Deconstruction of lignocellulosic biomass to fuels and chemicals. Annual review of chemical and biomolecular engineering, 2011. 2: p. 121-145.

[9] Payne, C.M., et al., Fungal cellulases. Chemical reviews, 2015. 115(3): p. 1308-1448.

[10] Saibi, W. and A. Gargouri, Mastery of cultural conditions and physico-chemical properties improves the production and the catalytic efficiency of bglG. Applied Biochemistry and Biotechnology, 2013. 170(6): p. 1525-1532.

[11] Saibi, W., B. Amouri, and A. Gargouri, Purification and biochemical characterization of a transglucosilating beta-glucosidase of Stachybotrys strain. Appl Microbiol Biotechnol, 2007. 77(2): p. 293-300.

[12] Saibi, W. and A. Gargouri, Purification and biochemical characterization of an atypical β-glucosidase from Stachybotrys microspora. Journal of Molecular Catalysis B: Enzymatic, 2011. 72(3-4): p. 107-115.

[13] Saibi, W. and A. Gargouri, Hydroxyl distribution in sugar structure and its contributory role in the inhibition of Stachybotrys microspora β-glucosidase (bglG). Carbohydrate Research, 2011. 346(13): p. 1848-1854.

[14] Ben Hmad, I., et al., Medium initial pH and carbon source stimulate differential alkaline cellulase time course production in Stachybotrys microspora. Appl Biochem Biotechnol, 2014. 172(5): p. 2640-9.

[15] Amore, A., S. Giacobbe, and V. Faraco, Regulation of cellulase and hemicellulase gene expression in fungi. Curr Genomics, 2013. 14(4): p. 230-49.

[16] Amouri, B. and A. Gargouri, Characterization of a novel β-glucosidase from a Stachybotrys strain. Biochemical Engineering Journal, 2006. 32(3): p. 191-197.

[17] Hayashi, T., et al., Cellulose metabolism in plants. International review of cytology, 2005. 247: p. 1-34.

[18] Gil-Amado, J.A. and M.C. Gomez-Jimenez, Transcriptome analysis of mature-fruit abscission control in olive. Plant and Cell Physiology, 2013: p. pcs179.

[19] Agustí, J., et al., Comparative transcriptional survey between laser-microdissected cells from laminar abscission zone and petiolar cortical tissue during ethylene-promoted abscission in citrus leaves. BMC Plant Biology, 2009. 9(1): p. 127.

[20] Meir, S., et al., Microarray analysis of the abscission-related transcriptome in the tomato flower abscission zone in response to auxin depletion. Plant Physiology, 2010. 154(4): p. 1929-1956.

[21] Gonzalez-Carranza, Z.H. and J.A. Roberts, Ethylene and cell separation processes. Annual Plant Reviews, The Plant Hormone Ethylene, 2012. 44: p. 246.

[22] Pilling, E. and H. Höfte, Feedback from the wall. Current opinion in plant biology, 2003. 6(6): p. 611-616.

[23] Dashtban, M., H. Schraft, and W. Qin, Fungal bioconversion of lignocellulosic residues; opportunities & perspectives. International Journal of Biological Sciences, 2009. 5(6): p. 578.

[24] Hilden, L. and G. Johansson, Recent developments on cellulases and carbohydrate-binding modules with cellulose affinity. Biotechnology letters, 2004. 26(22): p. 1683-1693.

[25] Shoseyov, O., Z. Shani, and I. Levy, Carbohydrate binding modules: biochemical properties and novel applications. Microbiology and Molecular Biology Reviews, 2006. 70(2): p. 283-295.

[26] Azriel-Rosenfeld, R., M. Valensi, and I. Benhar, A human synthetic combinatorial library of arrayable single-chain antibodies based on shuffling in vivo formed CDRs into general framework regions. Journal of molecular biology, 2004. 335(1): p. 177-192.

[27] Nahary, L. and I. Benhar, Design of a human synthetic combinatorial library of single-chain antibodies, in Therapeutic Antibodies. 2009, Springer. p. 61-80.

[28] Saibi, W. and A. Gargouri, Cellobiose dehydrogenase influences the production of S. microspora beta-glucosidase. World J Microbiol Biotechnol, 2012. 28(1): p. 23-9.

[29] Saibi, W. and A. Gargouri, Hydroxyl distribution in sugar structure and its contributory role in the inhibition of Stachybotrys microspora beta-glucosidase (bglG). Carbohydr Res, 2011. 346(13): p. 1848-54.

[30] Branden, C.I., Introduction to protein structure. 1999: Garland Science.

[31] Copeland, R.A., Enzymes: a practical introduction to structure, mechanism, and data analysis. 2004: John Wiley & Sons.

[32] Sikorski, P., et al., Serratia marcescens chitinases with tunnel-shaped substrate-binding grooves show endo activity and different degrees of processivity during enzymatic hydrolysis of chitosan. Biochemistry, 2006. 45(31): p. 9566-9574.

[33] Spezio, M., D.B. Wilson, and P.A. Karplus, Crystal structure of the catalytic domain of a thermophilic endocellulase. Biochemistry, 1993. 32(38): p. 9906-9916.

[34] Abdeljalil, S., et al., Improvement of cellulase and xylanase production by solid-state fermentation of Stachybotrys microspora. Biotechnol Appl Biochem, 2013.

[35] Doi, R.H. and A. Kosugi, Cellulosomes: plant-cell-wall-degrading enzyme complexes. Nature Reviews Microbiology, 2004. 2(7): p. 541-551.

[36] Chang-Jun, J., L. Yuan-Hua, and F. Wan-Ping, cDNA cloning and prokaryotic expression of β-glucosidase in tea plant [Camellia sinensis (L.) O. Kutze]. Chinese Journal of Agricultural Biotechnology, 2005. 2(02): p. 107-111.

[37] Juhász, T., Enzymes for improved hydrolysis of lignocellulosics, 2005, Thesis: Budapest University of Technology and Economics.

[38] Ilmen, M., et al., Regulation of cellulase gene expression in the filamentous fungus Trichoderma reesei. Applied and Environmental Microbiology, 1997. 63(4): p. 1298-1306.

[39] Wulff, G., Enzyme-like catalysis by molecularly imprinted polymers. Chemical reviews, 2002. 102(1): p. 1-28.

[40] Burns, K.L. and S.W. May, Separation methods applicable to the evaluation of enzyme–inhibitor and enzyme–substrate interactions. Journal of Chromatography B, 2003. 797(1): p. 175-190.

[41] Balandrin, M.F., et al., Natural plant chemicals: sources of industrial and medicinal materials. Science, 1985. 228(4704): p. 1154-1160.

[42] DeMarco, M.L. and R.J. Woods, Structural glycobiology: a game of snakes and ladders. Glycobiology, 2008. 18(6): p. 426-440.

[43] Javed, M.R., et al., Cost-efficient entrapment of β-glucosidase in nanoscale latex and silicone polymeric thin films for use as stable biocatalysts. Food Chemistry, 2016. 190: p. 1078-1085.

[44] Krisch, J., et al., Characteristics and potential use of β-glucosidases from Zygomycetes. Current research, technology and education topics in applied microbiology and microbial biotechnology, 2010.

[45] Papalazaridou, A., L. Charitidou, and A. Sivropoulou, β-Glucosidase enzymatic activity of crystal polypeptide of the Bacillus thuringiensis strain 1.1. Journal of endotoxin research, 2003. 9(4): p. 215-224.

[46] Rajoka, M.I., F. Jalal, and S.A. Bukhari, Enhanced sporulation and toxin production by a mutant derivative of Bacillus thuringiensis. African Journal of Biotechnology, 2012. 11(100): p. 16607-16614.

[47] Gill, S.R., et al., Metagenomic analysis of the human distal gut microbiome. science, 2006. 312(5778): p. 1355-1359.

[48] Greimel, P., et al., Iminosugars and relatives as antiviral and potential anti-infective agents. Current topics in medicinal chemistry, 2003. 3(5): p. 513-523.

[49] Morant, A.V., et al., β-Glucosidases as detonators of plant chemical defense. Phytochemistry, 2008. 69(9): p. 1795-1813.

[50] Morant, A.V., et al., The β-glucosidases responsible for bioactivation of hydroxynitrile glucosides in Lotus japonicus. Plant physiology, 2008. 147(3): p. 1072-1091.

[51] Morant, A., et al., The β-Glucosidases Responsible for Bioactivation of Hydroxynitrile Glucosides in Lotus japonicus. Plant physiology, 2010. 152(4).

[52] Lechtenberg, M., Cyanogenesis in higher plants and animals. eLS, 2011.

[53] Chapelle, A., et al., Impact of the absence of stem-specific β-glucosidases on lignin and monolignols. Plant physiology, 2012. 160(3): p. 1204-1217.

[54] Abdeljalil, S., et al., Investigations on hydrolytic activities from Stachybotrys microspora and their use as an alternative in yeast DNA extraction. Applied biochemistry and biotechnology, 2014. 172(3): p. 1599-1611.

[55] Saibi, W. and A. Gargouri, Purification and biochemical characterization of an atypical β-glucosidase from Stachybotrys microspora. Journal of Molecular Catalysis B: Enzymatic, 2011. 72(3): p. 107-115.

[56] Amyot, A., Les additifs pour le foin et l'ensilage: mode d'action et recommandations d'utilisation pour chaque type de produit. 2003.