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A REVIEW OF NATURAL AND ENGINEERED ENZYMES INVOLVED IN BIOETHANOL PRODUCTION

By

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A Review of natural and engineered enzymes involved in bioethanol production.

Alternative petroleum-derived fuels, such as biofuels, is another form to decrease the dependence of non-renewable energy. The most promising alternative energy is cellulosic ethanol because of the abundance of cellulose and the overall lack of concern for the food-versus-fuel dilemma.

In order to produce ethanol from cellulosic materials, pretreatment is required to "open" the lignocellulosic matrix and make cellulose more susceptible to enzymatic degradation. Enzymatic hydrolysis of lignocellulose is an important area of research due to the absence of negative effects in downstream processes in comparison with acid hydrolysis. Both natural enzymes and engineered enzymes can be used in the process of ethanol production. Natural enzymes are found either individually or as a part of a complex known as cellulosome. Such complexes are the focus of many studies due to the efficiency in the degradation of cellulose. Research in enzymatic engineering is being done in order to mimic these natural systems. Engineered individual enzymes are also used to improve the properties of the enzymes found in nature. Enzymes can be engineered by rational design or directed evolution. Directed evolution is the most efficient technology, since it only requires the knowledge of protein sequences. However, this approach also possesses some limitations. A combination of both methods or a "semi-rational" approach is perhaps the best option to develop higher performance lignocellulolytic enzymes.

Many advances regarding engineering of lignocellulolytic enzymes have been made in the last past years. Further research, however, is required in the development of enzymes systems and enzyme industrial testing to establish cellulosic bioethanol as main substitute for petroleum-derived fuel energy.

Chairperson or Co-Chairperson: Ceballos, Michael

INTRODUCTION

The interest in alternative energies has increased in the past years due to the unsustainable use of natural resources and their growing demand. Biofuels, which can be made from lipid-rich feedstocks (biodiesel) or carbohydrate-rich feedstocks (bioethanol), is one such form energy. Biofuels are considered renewable and, in many cases, sustainable and environmentally-friendly. Although bioethanol possesses numerous advantages, there are still some limitations in commercial production that prevent it from being cost competitive and the liquid fuel of choice. These limitations include: the use of land and water resources to produce corn- and sugarcane-based bioethanol (i.e., first generation biofuels) that competes with food crop production and the inefficient conversion of feedstock molecular substrates to fermentable sugars in cellulosic ethanol (i.e., second generation bioethanol). Current research is focused on overcoming these limitations. Since cellulose is the most abundant polymer on Earth and first generation bioethanol production creates a *food-versus-fuel* dilemma, cellulosic ethanol may be the most promising alternative liquid fuel option if production process bottlenecks can be resolved.

Pretreatment of cellulosic feedstock is typically required to expand or "loosen up" the lignocellulosic matrix. At the molecular level this entails: breaking hydrogen bonds between lignin and holocellulose; disrupting hydrogen bonding within holocellulose; lysing covalent bonds that stabilize lignin; and, destabilizing the crystalline of cellulose to render it more susceptible to degradation by sugar reducing enzymes. Depolymerization of molecular substrates (e.g., cellulose) from pretreated lignocellulosic materials to generate simpler fermentable, sugars (e.g., monosaccharides) is often achieved by one of two different sugar reduction processes: enzymatic hydrolysis; or, acid hydrolysis. Acid hydrolysis is mainly performed via sulfuric acid; however, due to negative effects in downstream processes, the use of this technology is limited. Using hydrolytic enzymes from microorganisms (i.e., bacteria and fungi) as a substitute for chemical approaches is technology continuously in development and an area of intense research. Enzymatic degradation of lignocellulose is performed using a variety of enzymes in specific ratios or sequences so that biomass deconstruction and generation of fermentable sugar may be achieved in an efficient manner. Multi-enzyme systems are designed to optimize synergistic interactions between different classes of enzymes during this process. In general, enzymes used in this process are referred to as "lignocellulolytic enzymes". Most are derived from natural systems and each class of enzymes possesses unique features including structural-functional modularity. Optimization of enzyme-mediated processes is particularly important if bioethanol is to become economically viable. Either enzymes found in nature or engineered enzymes can be utilized in industrial-scale bioethanol production processes. Attempts to mimic the activity of naturally-occurring extracellular macromolecular complexes called cellulosomes have been undertaken,

because of the efficiency by which cellulosomes degrade cellulosic biomass in nature. This has spawned the development of enzyme cocktails and engineered platform systems.

The goal of this article is to provide a review of the enzymes found in nature as well as engineered enzymes that are used in the production of bioethanol with focus on their respective modes of action and a description of the most important multienzyme systems found in nature are discussed, including the cellulosome of C. thermocellum.

NATURAL ENZYMES USED TO CONVERT FEEDSTOCK TO SUBSTRATE

Mode of Action of Primary Lignocellulolytic Enzymes

To use cellulosic material as feedstock for bioethanol production, chipped or ground biomass is typically pretreated to facilitate enzyme access to long chain carbohydrates (e.g., cellulose), which are the macromolecules that are reduced to fermentable sugar for conversion to ethanol. Given the heterogeneous nature of lignocellulose, it is highly recalcitrant even with pretreatment. Numerous methods have been developed for degrading lignocellulose to expose polysaccharides. Likewise, numerous approaches have been developed to reduce these macromolecular substrates. Those approaches that rely on enzymes produced by microorganisms can be highly efficient. Both multi-domain enzymes and enzyme complexes (e.g., mini-cellulosomes) have been applied. One useful feature of many lignocellulolytic enzymes (and their complexes) is innate modularity. In addition to a catalytic core region, many cellulolytic enzymes possess non-catalytic domains. Two notable domains include carbohydrate-binding modules (CBMs) and dockerin domains. CBMs facilitate interactions between enzymes and their respective carbohydrate substrates (Tomme et al., 1988; Tomme et al., 1998; Boraston et al., 1999; Gilbert et al., 2013). Various studies have demonstrated CBMs enhance enzymatic activity against recalcitrant substrates (Black et al., 1996; Bolam et al., 1998; Carrard et al., 2000; Mello and Polikarpov, 2014). Dockerin domains on cellulolytic enzymes from some species of microorganisms mediate cohesin–dockerin interactions, associating the enzymes with larger macromolecular complexes. These complexes, or *cellulosomes*, are found naturally at the cell membrane-cell wall structure of many cellulolytic microorganisms (see Fontes and Gilbert, 2010). Lignocellulolytic enzymes may be generally categorized as: cellulases, hemicellulases, ligninolytic enzymes and pectinases. This section provides a review of primary lignocellulolytic enzymes and their respective functions within natural cellulosomes.

- Cellulases

Cellulases are glycosyl or glycoside hydrolases (GHs) that catalyze cellulolysis, the cleaving of glycosidic bonds in cellulose. The enzyme-mediated cleavage of β-1,4-glycosidic bonds in cellulose occurs via acid hydrolysis, using a proton donor and a nucleophile or base. The products of acid hydrolysis either result in an inversion or retention (via single or double replacement, respectively) of the anomeric configuration of the carbon-1 (C1) at the reducing end (see Koshland, 1953; see Vocadlo and Davies, 2008). In macromolecular complexes, such as in a naturally-occurring cellulosome, cellulolytic enzymes act in a synergistic manner (Wood and McCrae, 1979; Lamed et al., 1983b; Fierobe et al., 2001). Synergism is a phenomenon that results in a mutual increase in the efficiency of action using two or more components in a system. Cellulolytic enzyme synergism can be measured qualitatively and quantitatively; however, predicting synergistic effects of novel combinations of enzymes either free in solution or bound in an artificial cellulosome has proven challenging and is the subject of intense investigation. According to studies on fungi (Selby and Maitland, 1967; Wood and McCrae, 1972; Berghem et al., 1976; Wood and McCrae, 1978; Mandels and Reese, 1999), bioconversion of polysaccharide substrates into simple fermentable sugars requires synergistic interactions of at least three types of enzymes: endoglucanases, cellobiohydrolase, and β-glucosidases. Most of these components are glycoproteins and each presents isoenzymes in natural systems (Wood and McCrae, 1972; Gilkes et al., 1984; Mihoc and Kluepfel, 1990; Jimenéz-Zurdo et al., 1996; Igual et al., 2001; Wei et al., 2005; Begum and Absar, 2009; Khalili et al., 2011). Functionally, cellulases may be categorized into groups based on the type of reaction catalyzed: carbohydrases (including, endoglucanases, exoglucanases and cellobiases); oxidative cellulases (e.g., cellobiose dehydrogenase); and, phosphorylases (i.e., cellobiose phosphorylase and cellodextrin phosphorylase).

- Carbohydrases

Carbohydrases are GHs that hydrolyze the β-1,4-glycosidic bonds of cellulose or cello-oligosaccharides, leading to the formation of short cello-oligosaccharides (cellodextrins) and glucose (Lombard et al., 2014; CAZy, 2015). There are three types of carbohydrases: endoglucanases or endocellulases (EGs); exoglucanases or exocellulases (EXs); and cellobiases, β-glucosidases or β-D-glucoside glucohydrolases (βGs).

EGs are 1,4-β-D-glucan-4-glucanhydrolases that disrupt bonds at random internal sites in the cellulose polysaccharide chain producing oligosaccharides of various lengths. The EGs produce

new chain ends (*see* Figure 10). EGs that do not feature CBMs hydrolyze at amorphous internal sites within the cellulose chain (Rabinovich et al., 1982; Stahlberg et al., 1988; Henriksson et al., 1999; Karlsson et al., 2002). EGs featuring CBMs can also hydrolyze cellulose chains at crystalline internal regions (Tilbeurgh et al., 1986; Gilkes et al., 1988; Tomme et al., 1988; Wang et al., 2012e). Furthermore, EG cellulolysis generates new chain ends for cellobiohydrolase (CBH) activity (Wood and McCrae, 1972; Berghem and Pettersson, 1973; Henrissat et al., 1985). When an enzyme does not readily release a large molecular substrate and catalyzes multiple reactions prior to dissociating from the substrate, it is considered to be "processive". Although processivity is typical of CBHs, some EGs also hydrolyze cellulose processively (Reverbel-Leroy et al., 1997; Irwin et al., 1998; Gilad et al., 2003; Cohen et al., 2005; Zverlov et al., 2005a; Zheng and Ding, 2013). EGs belong to GH families 5, 6, 7, 9, 12, 44, 45, 48, 51, 74 and 124 (Lombard et al., 2014; CAZy, 2015). All well-studied processive EGs are part of the GH-9 family, which includes most plant cellulases, some animal cellulases, and many bacterial cellulases. Surprisingly, very few fungal cellulases are included within the GH-9 family of EGs. Processive EGs from GH-9 family feature CBMs of the family 3c. These EG CBMs are positioned at the C terminus of the enzyme's catalytic domain (Sakon et al., 1997). They are required and responsible for processivity in this class of enzyme (Irwin et al., 1998; Gilad et al., 2003). Additionally, a new kind of processive EG has been reported recently that belongs to the GH-5 family (Cohen et al., 2005; Watson et al., 2009; Zheng and Ding, 2013).

EXs hydrolyze 1,4-β-D-glycosidic linkages in cello-oligosaccharides. Specifically, they cleave from the reducing or nonreducing ends of chains formed by EGs activity. These EXs act in a processive manner due to their shaped tunnel active sites (Rouvinen et al., 1990; Divne et al., 1994; Parsiegla et al., 1998). Several studies indicate that select EXs, such as CBH, are also capable of cleaving internal glycosidic bonds (Stahlberg et al., 1993; Armand et al., 1997; Boisset et al., 2000). There are two main groups of EXs: cellodextrinases and cellobiohydrolases (CBHs). Cellodextrinases, also known as 1,4-β-D-glucan glucanohydrolases and exo-1,4-β-glucosidase, liberate D-glucose from cellodextrins and cellulose (Barras et al., 1969). They belong to the GH families 1, 3, 5, 9 (Lombard et al., 2014; CAZy, 2015). CBHs (1,4-β-D-glucan cellobiohydrolases) liberate D-cellobiose from cellulose chain ends produced by EGs and from crystalline cellulose (Kleman-Leyer et al., 1996; Igarashi et al., 2009; Liu et al., 2011) while CBHII additionally releases D-cellobiose from amorphous cellulose (Koivula et al., 1998) (*see* figure 10). CBHI works processively from the reducing end of cellulose and CBHII works processively from the nonreducing end of cellulose (Fägerstam and Pettersson, 1980; Arai et al., 1989; Barr et al., 1996; Saharay et al., 2010). CBHIIs are grouped into GH families 5, 6, 9, while most of CBHIs belongs to GH families 7 and 48 (Lombard et al., 2014; CAZy, 2015). Due to enzyme processivity and large adsorption ability onto the insoluble cellulose substrates, CBH kinetics deviate from the Michaelis-Menten model of enzyme kinetics and exhibit fractal and "local jamming" effects (Xu and Ding, 2007; Igarashi et al., 2011; Kamat et al., 2013).

βGs hydrolyze the β-1,4-D-glycosidic bonds at the non-reducing ends of soluble cellodextrins and cellobiose to release monomeric β-glucose (Freer, 1993). Unlike other carbohydrases, βGs generally lack distinct CBMs and are therefore not modular in nature. Unlike the majority of biomass degrading enzymes, βGs can be studied using traditional kinetic models primarily due to they act by binding to soluble substrate (Kempton and Withers, 1992; Chauve et al., 2010). βGs serve an important role in the multi-enzyme system synergy by increasing glucose yield and minimizing inhibition of cellulases by cellobiose (Berlin et al., 2005a; Chir et al., 2011). Cellobiose is a strong inhibitor of CBH and EG (Holtzapple et al., 1990; Gusakov and Sinitsyn, 1992; Zhao et al., 2004; Andrić et al., 2010; Teugjas and Valjamae, 2013). Studies show that cellobiose inhibits cellulases 14 times more than glucose (Holtzapple et al., 1984). βGs are classified into GHs families 1, 3, 5, 9, 30, 116 (Lombard et al., 2014; CAZy, 2015).

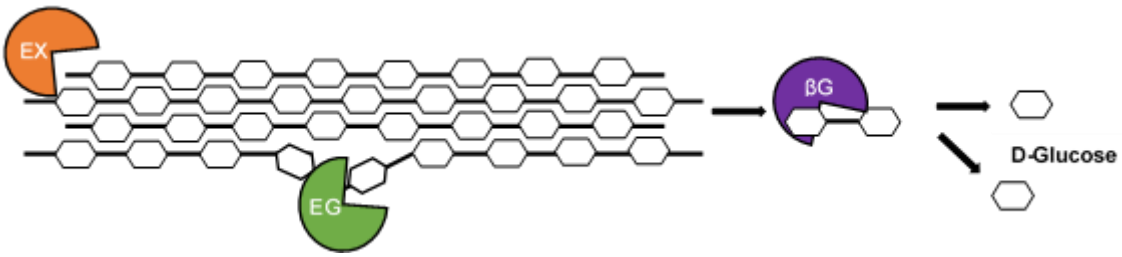

Figure 10. Schematic graphic of cellulose structure and mode of action of cellulolytic enzymes EX, EG, and βG leading to the formation of D-glucose. *Figure adopted from* (van den Brink and de Vries, 2011).

Different EGs possess different mechanisms ("inverting" for GH6, 9, 45, and 48 EGs; "retaining" for GH5, 7, 12 EGs. This EG "plurality" may be in relation with different EGs' side-activities on hemicellulose in deconstructing complex lignocellulose materials (Vlasenko et al., 2010), or synergism between processive and conventional EGs (Tuka et al., 1992; Qi et al., 2007).

- Oxidoreductive Cellulases

For decades, researchers have suggested that there exists an additional non-hydrolytic factor that makes biomass less recalcitrant and more susceptible to enzymatic attack (Reese et al., 1950). This includes a proposed oxidative mechanism for the initiation of cellulose degradation (Eriksson et al., 1974). Subsequently, it was confirmed that cellulose oxidases disrupt cellulose structure via oxidation thus, increasing substrate access to cellulase action (Forsberg et al., 2011; Quinlan et al., 2011). Although these enzymes occur in relatively low concentrations in natural systems, they play a central role in the cellulase systems of both aerobic fungi and bacteria, which degrade

cellulose (Harris et al., 2010; Forsberg et al., 2011). Interestingly, cellulose oxidases are completely absent in anaerobic cellulase complexes.

Oxidoreductive enzyme systems are commonly composed of cellobiose dehydrogenase (CDH), cellobiose quinone oxidoreductase (CBQOR), lactonase, glucose oxidase, and/or polysaccharide monooxygenases (PMOs) (*see* figure 11).

CDH, also known as cellobiose oxidoreductase (CBOR) or cellobiose oxidase (CBO), was discovered in 1974 and originally named CBQOR (Westermark and Eriksson, 1974a; Westermark and Eriksson, 1974b). The enzyme is formed by one flavin adenine dinucleotide (FAD) and one heme as prosthetic groups (Ayers et al., 1978; Morpeth, 1985). CBQOR is the term now used for a catalytic active fragment of CDH which lacks the heme group, is produced by posttranslational proteolytic cleavage, and has similar catalytic properties to CDH (Henriksson et al., 1991; Samejima and Eriksson, 1992; Wood and Wood, 1992; Raíces et al., 2002). CDH is the only known example of secreted flavocytochrome and is found in most wood-degrading fungi (see Zamocky et al., 2006). They catalyze the reducing end oxidation of cellobiose, cellodextrins, lactose, and maltodextrins or other oligosaccharides to the corresponding lactones using a wide spectrum of electron acceptors including quinones, phenoxyradicals, Fe^{3+}, Cu^{2+} and triiodide (see Henriksson et al., 2000). These lactones are converted into aldonic acids by spontaneous or enzymatic hydrolysis with lactonase (Brodie and Lipmann, 1955; Beeson et al., 2011). The biological role of CDH is not fully understood yet, but the research done over the last 50 years indicates its participation in the deconstruction of lignocellulose components (cellulose, hemicellulose and lignin) by generating hydroxyl radicals. The enzyme has the ability to reduce Fe^{3+} to Fe^{2+}, which together with hydrogen peroxide (H_2O_2), produces hydroxyl radicals via Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$) (Kremer and Wood, 1992a; Kremer and Wood, 1992b). Also, CDH diminishes the end-product inhibition of cellulases by removal of cellobiose (Ayers et al., 1978; Igarashi et al., 1998). In addition, recent evidence suggests the participation of the enzyme in the transfer of electrons to members of the PMOs to oxidatively breakdown plant-biomass constituents (Phillips et al., 2011; Sygmund et al., 2012; Vu et al., 2014). CDH are classified into auxiliary activity family 3 (Lombard et al., 2014; CAZy, 2015)

Lactonase, also known as a gluconolactonase, aldonolactonase or D-glucono-1,5-lactone lactonohydrolase, catalyzes the hydrolysis of different types of hexose-1,5-lactones to their corresponding aldonic acids (Brodie and Lipmann, 1955; Beeson et al., 2011). It is present in Novozyme 188, a commercial preparation based on enzymes from *Trichoderma reesei* and *Aspergillus niger* (Bruchmann et al., 1987). It can also promote cellulolysis by removing lactones, which are inhibitors of cellulases (Bruchmann et al., 1987; Verma et al., 2011; Rouyi et al., 2014).

Glucose oxidase, also known as notatin, is an oxido-reductase that mainly catalyzes the oxidation of glucose to H_2O_2 and D-glucono-δ-lactone which hydrolyses spontaneously to gluconic acid (Müller, 1928; Bentley and Neuberger, 1949). It is also considered integral components of cellulase, as it relieves inhibition of cellulases by glucose (Holtzapple et al., 1984; Stutzenberger, 1986; Holtzapple et al., 1990; Xiao et al., 2004; see Andrić et al., 2010; Hsieh et al., 2014). Glucose oxidase belongs to auxiliary activity 3 (Lombard et al., 2014; CAZy, 2015).

PMOs were first discovered in 2010 by G. Vaaje-Kolstad (Vaaje-Kolstad et al., 2010). They are copper-dependent metalloenzymes that oxidatively cleave glycosidic bonds at the surface of recalcitrant cellulose structures (Forsberg et al., 2011; Phillips et al., 2011; Quinlan et al., 2011). For efficiency, PMOs require molecular oxygen (O_2) and an electron donor, such as CDH (Phillips et al., 2011; Sygmund et al., 2012; Vu et al., 2014). PMOs introduce O_2 to C−H bonds adjacent to the glycosidic linkage, which leads to the removal of the adjacent carbohydrate moiety (Phillips et al., 2011; Beeson et al., 2012). PMOs can be subdivided into at least three types based on structure and substrate specificity. Type 1 PMOs generate products that are oxidized at C1. Type 2 PMOs generate products oxidized at the non-reducing end of C4. Type 3 PMOs exhibit weaker specificity and release oxidized products from both reducing and non-reducing ends (Beeson et al., 2012; Li et al., 2012). PMOs are classified into auxiliary activity families 9 and 10 (Lombard et al., 2014; CAZy, 2015).

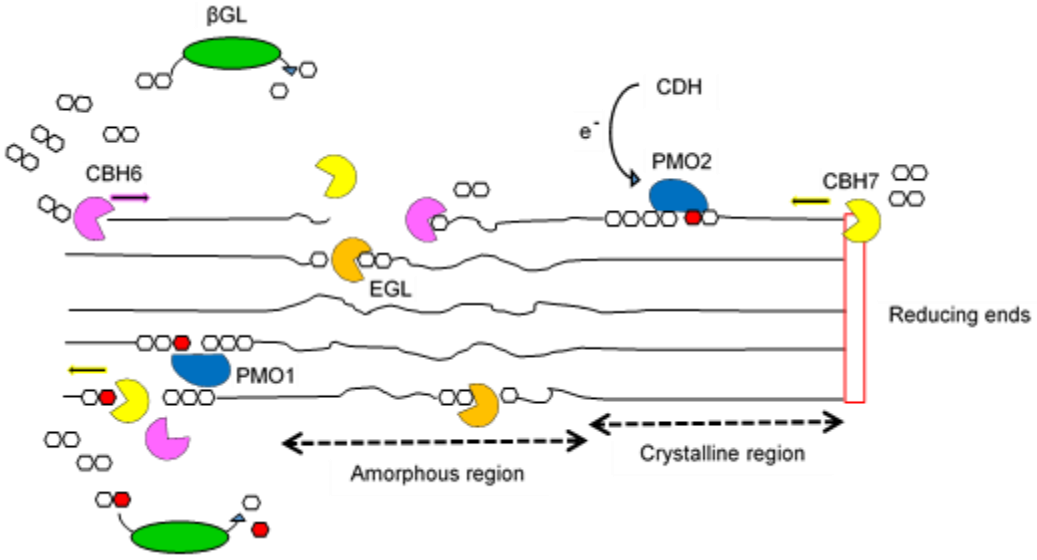

Figure 11. Schematic representation of the enzymatic degradation of cellulose, involving CBH, βG, EG, Type 1 PMOs (PMO1), Type 2 PMOs (PMO2), and CDH. *Figure adopted and modified from* (Dimarogona et al., 2012).

- Phosphorylases

Phosphorylases reduce cellobiose and cellodextrins to glucose using phosphates instead of water (Ayers, 1959; Alexander, 1961; Sheth and Alexander, 1967). Recall that cellobiose and cellodextrin are formed during the enzymatic degradation of cellulose by EGs and EXs. Since phosphorolytic enzymes are located inside cells, organisms transport substrate saccharides from the extracellular matrix across the cell membrane. For example, the thermophile *Clostridium thermocellum* employs ATP-driven transport mechanisms to intake not only glucose, but also cellobiose and cellodextrin (Strobel et al., 1995). Phosphorolysis can then take place on these products of hydrolysis to assist cellulose breakdown. Although phosphorylases are not directly part of the cellulolytic pathway, phosphorolysis may accelerate the rate of overall cellulosic degradation when acting in concert with hydrolytic enzymes by removing inhibitory intermediary products such as cellobiose (Holtzapple et al., 1990; Gusakov and Sinitsyn, 1992; Zhao et al., 2004; see Andrić et al., 2010; Teugjas and Valjamae, 2013). Phosphorylysis is energetically advantageous. Phosphorolysis results in conservation of a portion of the energy from the cleaved glycosyl bond. Glucose-1-phosphate (G1P) leads to the formation of activated glucosyl molecules with the investment of only one additional ATP molecule for the uptake of either cellobiose or cellodextrin. Each glucose molecule produced via hydrolytic cleavage would require two ATPs - one ATP for transport and another for activation (Goldberg, 1975; Strobel et al., 1995). There are two general types of phosphorylases: cellodextrin phosphorylases (CDPs) and cellobiose phosphorylases (CBPs).

CDPs phosphorylate cellodextrins released by EGs to cellodextrin (N-1), where N is the number of glucose units in the chain, and G1P (Sheth and Alexander, 1967). CBPs phosphorylate cellobiose into glucose and G1P during the transport of cellobiose into the cell (Alexander, 1961) (*see* figure 12). Both enzymes are classified into GH family 94 (Lombard et al., 2014; CAZy, 2015)

1) Cellodextrin + Pi ⇌ cellodextrin (N-1) + G-1P
2) Cellobiose + Pi ⇌ glucose + G-1P

Figure 12. Chemical reaction catalyzed by CDP (1) and CBP (2).

- Hemicellulases

Hemicellulases are either GHs or carbohydrate esterases (CEs) that catalyze the hydrolysis and deacetylation of hemicelluloses, respectively (*see* table 7). The mode of action of hemicellulases varies with the type of enzyme (see Vocadlo and Davies, 2008; see Biely, 2012). The heterogeneity and organization of hemicellulose requires the concerted synergistic action of multiple enzymes for complete degradation. The deconstruction of the hemicellulose component in a feedstock exposes cellulose to cellulases and converts hemicellulose into usable saccharides. A principal component of hemicellulose is xylan (Timell, 1967). Therefore, xylanases are common enzymes used to breakdown hemicellulose. Several types of accessory enzymes also play important roles in degrading hemicellulose by acting on side chains of the heteropolymer to facilitate breakdown.

- Main Xylan Degrading Enzymes

Xylan is naturally heterogeneous. Its hydrolysis requires the action of complex enzyme systems. Microbial enzymes act in cooperative manner to convert xylan to its constituent simple sugars. The main enzymes involved are hydrolytic enzymes that hydrolyze β-1,4-xylosidic linkages. These enzymes are grouped into three classes: endo-1,4-β-xylanases (ENs), β-xylosidases (βXs), and exoxylanases (EXYs) (*see* figure 13).

ENs are GHs that hydrolyze β-1,4-xylose linkages in the interior of the heteroxylan backbone and generate xylooligosaccharides. EN action on substrate is not random but instead is determined by the chain length, the degree of branching, or the presence of specific substituents such as arabinofuranosyl groups (Li et al., 2000; von Gal Milanezi et al., 2012). ENs also play a role in lignin removal (Aracri and Vidal, 2011; Valenzuela et al., 2013). They deconstruct the xylan closely associated with lignin, which enhance the accessibility and extractability of lignin (Roncero et al., 2005). ENs are classified into families 5, 8, 10, 11, 30, 43 and 51 of GHs based on amino acid sequence similarities (Lombard et al., 2014; CAZy, 2015). The GH-10 and GH-11 ENs differ in substrate specificity. GH-10 ENs are capable of cleaving glycosidic linkages in the xylan main chain adjacent to substituents (atom or functional group in place of a hydrogen atom on a hydrocarbon), while GH-11 ENs preferentially cleave unsubstituted regions. As a result, GH-10 ENs release products that are shorter than the products of GH-11 EN action (Biely et al., 1997; Ustinov et al., 2008).

βXs release monomeric xylose from the non-reducing ends of xylooligomers and xylobiose produced by EN action on xylan. βXs have molecular weights >100 kDa and typically consist of two or more subunits (Matsuo and Yasui, 1984; Hebraud and Fevre, 1990; Eneyskaya et al., 2003; Eneyskaya et al., 2007). They have catalytic cores of the GH1, 3, 30, 39, 43, 51, 52, 54 and 116, 120 families (Lombard et al., 2014; CAZy, 2015). In general, βX activity on xylooligosaccharides decreases rapidly with increasing chain length (Van Doorslaer et al., 1985; Rasmussen et al., 2006). Many βXs exhibit α-L-arabinofuranosidase activity and some βXs reportedly have βG activity (Rodionova et al., 1983; Uziie et al., 1985; Xiong et al., 2007; Watanabe et al., 2015). Notably, most βXs are susceptible to xylose inhibition, which can significantly affect enzymatic efficiency under process conditions (Dekker, 1983; Poutanen, 1988; Herrmann et al., 1997; Saha, 2003b; Fujii et al., 2011; Kirikyali and Connerton, 2014). By splitting xylobiose, βXs relieve EN end product inhibition (see Sunna and Antranikian, 1997; Williams et al., 2000).

The EXYs are the most recent enzymes to be characterized in xylan degradation. Only a few of these EXYs have been reported and information on their catalytic properties is limited. What is known is that EXYs act on the xylan backbone from the reducing end to release xylose and short xylooligomers (Ganju et al., 1989; Kubata et al., 1994; Kubata et al., 1995; Usui et al., 1999; Honda and Kitaoka, 2004; Fushinobu et al., 2005; Tenkanen et al., 2013; Juturu et al., 2014). EXYs differ from βXs in that the former are inactive on xylobiose (Kubata et al., 1994; Kubata et al., 1995). EXYs can increase the rate of hydrolysis of xylan, since ENs would increase the ends available on the xylan backbone to the EXYs (Gasparic et al., 1995; Juturu et al., 2014). EXYs belongs to GH family 8 (Lombard et al., 2014; CAZy, 2015).

- Accessory Enzymes

Accessory enzymes either degrade the side chains of xylans (debranching enzymes) or act on the backbone chains of different kinds of hemicelluloses (backbone degrading enzymes). They are hydrolases or esterases.

Accessory xylanolytic enzymes

Accessory xylanolytic enzymes include: α-L-arabinofuranosidases (AFs); arabinoxylan arabinofuranohydrolases (AXAHs); endo-1,5-α-arabinanases cumulatively known as arabinases (AR); xylan α-D-glucuronidases or xylan α-1,2-D-glucuronidase (AgluAs); mannan endo-1,4-β-

mannosidase, 4-β-D-mannan mannanohydrolase, endo-1,4-mannanase or, simply, the β-mannanases (MANs); β-mannosidases (MNDs); α-galactosidases (AGL) and β-galactosidases (LACs), or, simply, the galactosidases; βGs; endo-β-1,4-galactanases (EG); xyloglucan-β-1,4-endoglucanases or xyloglucanase, cumulatively known as the xyloglucanendohydrolases (XGHs); α-D-xyloside xylohydrolase or, simpky, α-xylosidases (AXLs); α-fucosidases (AFUs); acetylxylan esterases (AXEs); ferulic acid esterases or feruloyl esterases, also known as, the cinnamoyl esterase hydrolyses (FAEs); *p*-coumaric acid esterases or p-coumaroyl esterase (PAE); glucuronyl esterases (GEs); and, acetyl mannan esterases (AME) (*see* figure 13).

Enzyme	Abbrev	Mode of Action
Hydrolases		
α-L-arabinofuranosidase	AF	non-reducing end of α- 1,2-, α-1,3-, α-1,5-linked arabinofuranosyl groups from arabinans, arabinoxylans, and arabinogalactans
α-fucosidase	AFU	L-fucose residues from xyloglucan branches
α-galactosidase	AGL	non-reducing end of α-linked D-non-reducing end galactose residues from xylan and galactomannans
α-D-glucuronidase	AgluA	non-reducing end of α-1,2-linked 4-O-methyl-D-glucuronic acid residues from glucuronoxylans
endo-1,5-α-arabinanase (arabinase)	AR	α(1→5) glycosidic bonds in arabinan
arabinoxylan arabinofuranohydrolase	AXAH	non-reducing end L-arabinofuranosyl groups from β-1,4-linked arabinoxylans
α-D-xyloside xylohydrolase or α-xylosidase	AXL	α-linked D-xylose residues from the xyloglucan backbone
β-glucosidase	βG	non-reducing end of β-D-glucosyl residues from glucomannan and galactoglucomannan oligosaccharides
β-xylosidases	βX	non-reducing ends of xylooligomers and xylobiose
endo-galactanase	EG	1,4-β-linked galactose residues in arabinogalactans
endo-1,4-β-xylanase	EN	β-1,4-xylose linkages in heteroxylan backbone
exo-β-1,4-xylanase	EXY	reducing end of xylan backbone
β-galactosidase	LAC	non-reducing end of β-linked D-galactose residues from xylan, xyloglucan, and galactoglucomannans
mannan endo-1,4-β-mannosidase, 1,4-β-D-mannan mannanohydrolase or endo-1,4-mannanase (β-mannanase)	MAN	β-1,4-linked bonds in mannan
β-mannosidase	MND	β-1,4-linked mannan oligosaccharides and mannobiose
xyloglucan-β-1,4-endoglucanase or xyloglucanase (xyloglucanendohydrolase)	XGH	1,4-beta-D-glucosidic linkages in xyloglucan
Esterases		
acetyl mannan esterase	AME	acetyl groups from galactoglucomannan
acetylxylan esterase	AXE	acetyl esters in xylan and xylooligomers

ferulic acid esterase or feruloyl esterase (cinnamoyl esterase hydrolases)	FAE	monomeric or dimeric ferulic acid from xylans
glucuronyl esterase	GE	4-O-methyl-D-glucuronic acid residues of glucuronoxylans
p-coumaric acid esterase or *p*-coumaroyl esterase	PAE	monomeric and dimeric p-coumaric acid

Table 7. Summary of lignocellulosic enzyme classes.

The AF catalytic domain belongs to the GH2, 3, 43, 51, 54, and 62 families of hydrolases (Lombard et al., 2014; CAZy, 2015). AF acts by cleaving the non-reducing end of α-1,2-, α-1,3- and α-1,5-linked L-arabinofuranosyl groups from hemicellulose, such as arabinoxylans or arabinogalactans (Saha and Bothast, 1998; Verbruggen et al., 1998b; Ahmed et al., 2013). This mode of action is effective in hydrolyzing hemicellulose side chains and disrupting structures anchored by α-glycosidic bonds. AXAHs are essentially AFs from the GH51 family of enzymes (Lombard et al., 2014; CAZy, 2015). However, AXAHs specifically remove the terminal non-reducing arabinofuranosyl residues from the 1,4-β-xylan backbone of arabinoxylans (Kormelink et al., 1991; Ferre et al., 2000; Lee et al., 2001). AFs and AXAHs also facilitate the disruption of lignin–carbohydrate binding at locations where arabinose residues are involved in lignin–hemicellulose ether bonds (Sun et al., 2005). Other AFs that exhibit βX or xylanase activity have also been described (Utt et al., 1991; Matte and Forsberg, 1992; Mai et al., 2000; Lee et al., 2003a). In addition to acting like other AFs, ARs with catalytic domains belonging to the GH43 family can also cleave internal α(1→5) glycosidic bonds in arabinan (Hong et al., 2009; Lombard et al., 2014; Shi et al., 2014; CAZy, 2015). AgluAs, of the GH67 and GH115 family of hydrolases, typically cleave α-1,2-glycosidic bonds of the 4-O-methyl-D-glucuronic/D-glucuronic acid residues from the terminal, non-reducing xyloses of glucuronoxylooligosaccharides or polymeric glucuronoxylan (Tenkanen and Siika-aho, 2000; Nurizzo et al., 2002; Ryabova et al., 2009; Lee et al., 2012; Lombard et al., 2014; Rogowski et al., 2014; CAZy, 2015). MANs cleave β-1,4-linked internal bonds in mannan backbone polymers producing new chain ends and releasing short β-1,4-manno-oligosaccharides (Mandels, 1965; Stålbrand, 1993; Katrolia et al., 2013). MANs belong to the GH5, GH26 and GH113 families (Lombard et al., 2014; CAZy, 2015). MNDs cleave β-1,4-linked manno-oligosaccharides and mannobiose from the degradation products of endomannases (e.g., MANs) producing mannose by acting at non-reducing terminal ends (Gübitz et al., 1996; Andreotti et al., 2005; Zhang et al., 2009). MNDs are GH1, 2 and 5 enzymes (Lombard et al., 2014; CAZy, 2015). Galactosidases are GHs which catalyze the hydrolysis of galactosides into monosaccharides. There are two types of galactosidases. AGLs, which belongs to GH 4, 27, 31, 36, 57, 97 and 110 families, release α-linked D-galactose residues from hemicellulose, eg., xylan or galactomannan by acting at the non-reducing terminal ends (Ademark et al., 2001; Lombard et al., 2014; CAZy, 2015). LACs, belonging to the GH1, 2, 35, 42 and 59 families (Lombard et al., 2014; CAZy, 2015), hydrolyze the non-reducing ends of β-D-galactose residues from

hemicellulose, eg., xylan, xyloglucan or galactoglucomannans (Sims et al., 1997). The βGs are also exo-type enzymes that remove the 1,4-β-D-glucopyranose units from non-reducing ends of oligosaccharides arising from the breakdown of glucomannan and galactoglucomannan by MAN (see Moreira and Filho, 2008). EGs, belonging to the GH53 family, also hydrolyze 1,4-β-linked galactose residues in arabinogalactans (Lombard et al., 2014; CAZy, 2015). XGHs hydrolyze fragmented xyloglucans into oligoxyloglucans and belong to GH 5, 9, 12, 16 and 44 families (Lombard et al., 2014; CAZy, 2015). AXLs release D-xylose residues with α-linkages from the non-reducing terminal of xylogluco-oligosaccharide (Ariza et al., 2011; Larsbrink et al., 2011). They belong to GH 31 family (Lombard et al., 2014; CAZy, 2015). AFUs, belonging to the GH29 and 95 families, release L-fucose residues from xyloglucan branches (Léonard et al., 2008; Lombard et al., 2014; CAZy, 2015). These represent the major hydrolases.

In addition to hydrolases acting on glycosidic linkages in hemicellulose, CEs catalyze the O-de- or N-deacylation of substituted saccharides. This only considers CEs in which sugars play the role of acid, such as in acetylated xylan. CEs can be grouped into different enzyme classes: AXEs, belonging to the CE1, 2, 3, 4, 5, 6, 7, and 12 families (Lombard et al., 2014; CAZy, 2015), hydrolyze acetyl ester bonds at the C-2, C-3 and C-4 positions of xylose in both xylan and xylooligomers (removing O-acetyl groups) (see Biely, 2012). FAEs, belonging to the CE1 family of esterases, hydrolyze hydroxycinnamoyl ester bonds liberating hydroxycinnamic acids, such as monomeric or dimeric ferulic acid (FA) (Lombard et al., 2014; CAZy, 2015). FAE action can target O2 or O5 on α-L-arabinoses's in xylans. Different FAEs exhibit different specificities driven by the nature of cinnamoyl substitution, which may either occur via hydroxylation or methoxylation, and/or the hemicellulose linkages (e.g., arabinose versus galactose ester bonds on xylans or on pectin) (see Benoit et al., 2008). The PAEs hydrolyze ester linkages between arabinose side chain residues of phenolic acids including monomeric and dimeric p-coumaric acid (PA) (Borneman et al., 1991). They are classified into the CE1 family (Lombard et al., 2014; CAZy, 2015). GEs belonging to the CE15 family (Lombard et al., 2014; CAZy, 2015) hydrolyze methyl ester bonds between 4-O-methyl-D-glucuronic acid residues of glucuronoxylans and aromatic alcohols of lignin (Špániková and Biely, 2006; Ďuranová et al., 2009). AMEs release acetyl groups from galactoglucomannan (see Shallom and Shoham, 2003) and belong to family CE16 family (Lombard et al., 2014; CAZy, 2015).

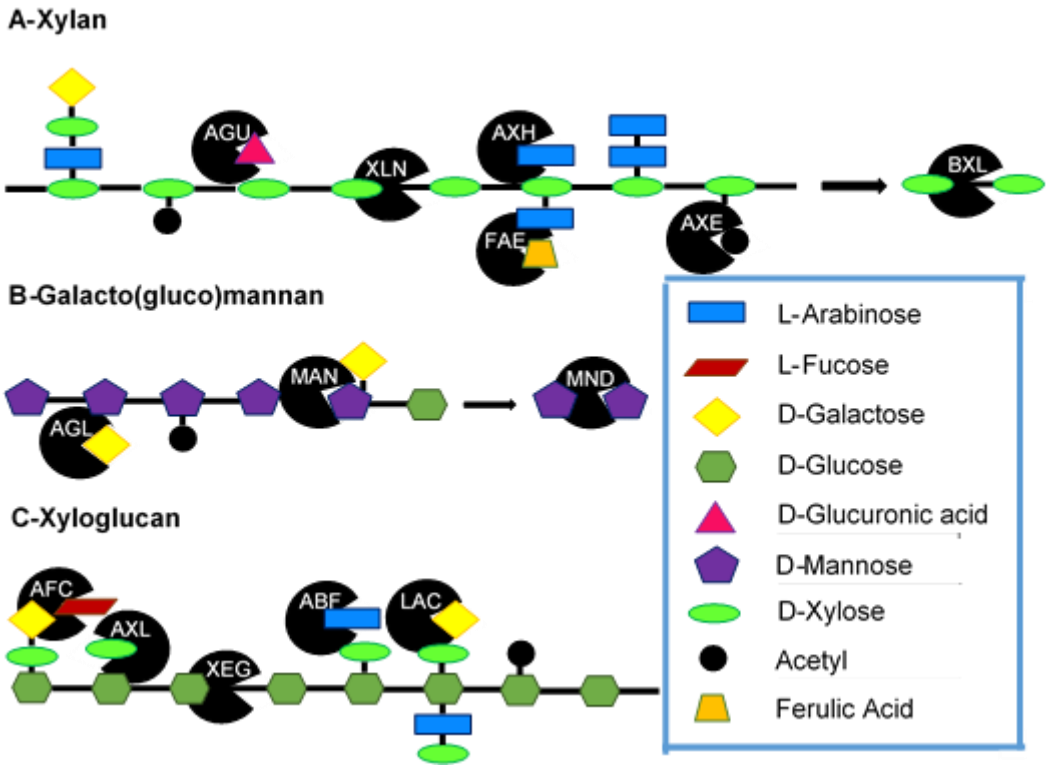

Figure 13. A–C Schematic representation of xylan, galacto(gluco)mannan, and xyloglucan; and mode of action of hemicellulolytic enzymes. *Figure adopted and modified from* (van den Brink and de Vries, 2011).

- Ligninolytic Enzymes

White rot fungi such as *Phlebia spp.* have the ability to effectively degrade lignin to CO_2 and H_2O so that carbohydrate polymers in plant cell walls can be used as carbon and energy sources (Fackler et al., 2006; Arora and Sharma, 2009). Notably, there are multiple studies on the degradation of lignin by white- and brown-rot fungi; however, fewer reports are available on lignin breakdown by soil bacteria (Crawford et al., 1983; Mercer et al., 1996; see Kirby, 2006; see Bugg et al., 2011). Two major families of oxidative enzymes (oxidoreductases) are involved in ligninolysis: peroxidases, including lignin peroxidases, also known as ligninase and diarylpropane oxygenase (LiPs), manganese-dependent peroxidases (MnPs), versatile peroxidases (VPs), and dye decolorizing peroxidase or also known as dyP-type peroxidases (DyPs), and laccases (Lacs). These enzymes catalyze a single-electron oxidation of lignin (transfer of one electron in each step from aromatic lignin components with low reduction potential to the high redox potential active site in the enzyme) that generates highly reactive non-specific free radicals (small agents), such as reactive oxygen species, which initiate lignin depolymerization by various non-enzymatic

reactions (Harvey et al., 1985; Schoemaker et al., 1985; see Hammel et al., 2002). In addition, some accessory enzymes, such as oxidases, are involved in the degradation of lignin by increasing the ligninolytic activity of principal enzymes. Although many enzymes involved in wood lignin degradation cannot penetrate the compact structure of woody tissues (Srebotnik et al., 1988; Flournoy et al., 1993; Blanchette et al., 1997), the enzymes can act at the surface of the cell wall producing low molecular mass agents (see Evans et al., 1994). These low molecular compounds can diffuse through the cell wall and initiate wood decay facilitating the penetration of lignin-degrading enzymes (Galkin et al., 1998).

- Peroxidases

Extracellular heme peroxidases belonging to the auxiliary activity family 2 (Lombard et al., 2014; CAZy, 2015) exhibit high potency in oxidative degradation of lignin and require extracellular H_2O_2 as an electron acceptor. Upon interaction with H_2O_2, these enzymes form highly reactive Fe^{+5}- or Fe^{+4}-oxo species (intermediates in catalytic reactions). These oxoferryl species remove electrons from lignin causing oxidation or radicalization, such in the case of LiPs. Regarding MnPs, an oxoferryl specie oxidazes Mn^{2+} to Mn^{3+}, which mediates lignin oxidation (see Wong, 2009) (*see* figure 14). The two main peroxidases LiP and MnP were discovered in the mid-1980s in *Phanerochate. chrysosporium* and exhibit high redox potential (Tien and Kirk, 1983; Kuwahara et al., 1984; Millis et al., 1989). Common peroxidases found in cellulose degradation systems include LiPs, MnPs, as well as the VPs and DyPs (described below).

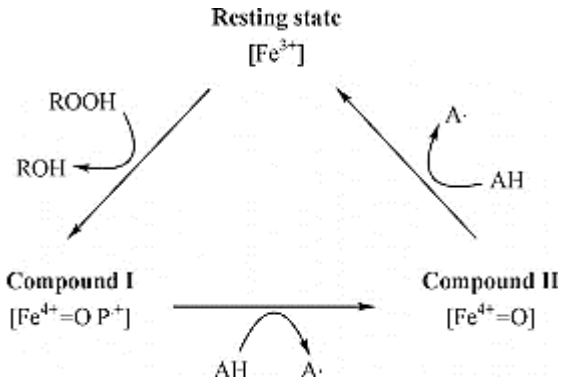

Figure 14. General catalytic cycle of heme-containing peroxidases: First, the resting state (Fe^{3+}) is involved in two-electron oxidation with H_2O_2 to form a Compound I oxo-ferryl intermediate (Fe^{4+}= $OP^{\cdot+}$). Then, Compound I oxidazes electron donor substrates (AH) by one-electron oxidation yielding Compound II (Fe^{4+}=O) and a substrate cation radical (A·). The last step implies another oxidation of substrate by Compound II subtracting one electron and consequently, returning the enzyme to the resting state (see Veitch, 2004). *Figure adopted from (Furukawa et al., 2014)*.

LiP was first discovered in *P. chrysosporium.* LiPs catalyze the H_2O_2-dependent oxidative depolymerization of lignin (Tien and Kirk, 1983; Tien and Kirk, 1984). In most fungi, LiP is present as a series of isoenzymes encoded by different genes (Glumoff et al., 1990; Johansson et al., 1993). LiPs are strong oxidants with higher redox potentials (>1 V) than those found in other types of peroxidases (Ward et al., 2003). This is because the porphyrin ring iron component in LiPs is more electron deficient than in classical peroxidases (Millis et al., 1989). LiPs are considered to be the most effective (and versatile) class of peroxidases. They can oxidize phenolic and non-phenolic compounds (Mester et al., 2001; Ward et al., 2001). LiP-catalyzed reactions include: (1) cleavage of C-C bonds; (2) cleavage of ether (C-O-C) bonds in non-phenolic aromatic substrates; (3) hydroxylation of benzylic methylene groups; (4) oxidation of benzyl alcohols to aldehydes or ketones; (5) phenol oxidation; and, (6) aromatic cleavage of non-phenolic lignin model compounds (Tien and Kirk, 1984; Hammel et al., 1985; Leisola et al., 1985; Renganathan et al., 1985; Renganathan et al., 1986; Umezawa et al., 1986). Note that LiP is too large to enter a plant cell; thus, LiP works on exposed regions of the lumen.

At the molecular level, MnPs are similar to the aforementioned LiPs. MnPs are extracellular heme enzymes that use manganese as a cofactor (Glenn and Gold, 1985; Paszczyński et al., 1986). MnPs were first discovered in *P. chrysosporium* (Kuwahara et al., 1984). The principal function of MnP is to oxidize Mn^{2+} to Mn^{3+} using H_2O_2 (Glenn et al., 1986). Mn^{2+} interacts with MnPs and H_2O_2 leading to the formation of a Mn^{3+}oxalate complexes. However, Mn^{2+} must be first chelated by organic acid chelators to stabilize the Mn^{3+} product. This process produces diffusible oxidizing chelates (Glenn and Gold, 1985; Glenn et al., 1986; Perez and Jeffries, 1992). Although Mn^{3+} is a strong oxidant that can leave the active center and oxidize phenolic compounds, it cannot attack non-phenolic units of lignin (Popp and Kirk, 1991). Resulting phenoxy-radicals undergo a variety of reactions leading to lignin depolymerization (Tuor et al., 1992). MnP can also oxidize non-phenolic lignin model compounds in the presence of Mn^{+2} via peroxidation of unsaturated lipids (Jensen et al., 1996; Kapich et al., 2005; Kapich et al., 2010). It has been suggested that white-rot fungi, which produce MnP and laccase, but not LiP, may produce mediators to enable MnP to cleave non-phenolic lignin substrates (Reddy et al., 2003).

VP from a white fungus (*Pleurotus eryngii*) was reported as a novel peroxidase possessing both MnP and LiP activity. VP oxidizes both phenolic and non-phenolic aromatic compounds, including veratryl alcohol and p-dimethoxybenzene. VP is also able to oxidize Mn2+ like MnPs (Martinez et al., 1996; Martinez et al., 1996; Camarero et al., 1999; Ruiz-Duenas et al., 1999). However, it possesses a high-redox potential for non-phenolic compounds similar to LiP (Camarero et al., 1999). VP is a heme-containing ligninolytic peroxidase with a unique hybrid molecular structure consisting of different active sites that mediate oxidation (Pérez-Boada et al., 2005; Ruiz-Duenas et al., 2009). VP can oxidize hydroquinone without exogenous H_2O_2 if Mn^{+2}

is available for the reaction. Chemical oxidation of hydroquinones promoted by Mn^{+2} may be important for the initial steps of wood biodegradation, since ligninolytic enzymes cannot penetrate unmodified wood cell walls (Gomez-Toribio et al., 2001).

DyPs were first described in fungi (Kim and Shoda, 1999). DyPs are also heme-containing peroxidases, which exhibit no primary sequence or structural similarities to other plant, bacterial, and fungal peroxidases, and perform better than other peroxidases in lower pH (Sugano et al., 1999; Sugano et al., 2007; Sugano, 2009). DyPs possess broad substrate specificity and oxidize all of the typical peroxidase substrates. DyPs can also oxidize high-redox potential synthetic dyes (i.e., anthraquinones), which are not converted by the other peroxidases (Kim et al., 1995; Kim and Shoda, 1999; Sugano et al., 2000; Liers et al., 2010; Santos et al., 2014). Although ligninolytic activity of DyPs has been reported, the actual physiological role of these peroxidases remains unclear. However, evidence suggest that some could be involved in lignin degradation (see Sugano, 2009; Adav et al., 2010; Ahmad et al., 2011; Salvachúa et al., 2013).

- Laccases

Lacs are glycosylated multi-copper phenoloxidases of the auxiliary activity family 1 found in plants, fungi, and bacteria (see Dwivedi et al., 2011; Lombard et al., 2014; CAZy, 2015). Lacs are widely distributed across the wood-degrading fungi (see Baldrian, 2006). Lacs do not require manganese or H_2O_2. They catalyze the single-electron oxidation of substrates through a concomitant four electron reduction of O_2 to H_2O (see Solomon et al., 1996; Messerschmidt, 1997; see Solomon et al., 2001). Lacs can directly oxidize phenolic components of lignin. Direct oxidation of phenolic lignin units generate phenoxy-free-radical products, which ultimately lead to polymer cleavage (Kawai et al., 1988). Substrates with high redox potential are not susceptible to Lac activity, since the enzyme possesses a relatively low redox potential ($\leq$0.8 V) (Reinhammar, 1972; Schneider et al., 1999; Johnson et al., 2003; Uzan et al., 2010). For example, non-phenonlics, which have a redox potential above 1.3V (Zweig et al., 1964), cannot be directly oxidized by Lacs. To degrade non-phenolic components, Lacs require the assistance of suitable mediators. Upon oxidation by Lacs, these low molecular weight compounds are converted to radicals and act as redox mediators that, in turn, oxidize other compounds that are not directly Lac substrates (Bourbonnais and Paice, 1990; Barreca et al., 2003; Cho et al., 2008). In bioethanol production, if Lacs are used directly as a biomass pretreatment, the addition of exogenous mediators, such as 2,29-azinobis(3-ethylbenzthiazoline-6-sulfonate) (ABTS) (Bourbonnais and Paice, 1990), may not be necessary, since natural mediators will likely be generated. For example, initial Lac activity on phenolic lignin units can result in the release of phenoxy radicals, which are natural mediators that oxidize more recalcitrant non-phenolic lignin moieties (d'Acunzo et al.,

2006; Nousiainen et al., 2009). It has been reported that this enzyme also possesses demethylating activity in lignin subunits (Trojanowski et al., 1966; Harkin and Obst, 1974; Ishihara and Miyaxaki, 1976; Leonowicz et al., 1979; Ander et al., 1983; Malarczyk et al., 2009) and lignin preparations (Ishihara and Miyaxaki, 1974; Ander and Eriksson, 1985; Crestini and Argyropoulos, 1998; Ibrahim et al., 2011). During demethylation, laccases act on aryl-O-alkyl C-O bonds.

- Accessory Enzymes and Mediators

There are some accessory enzymes and non-enzymatic components known as mediators that increase ligninolytic activity of principal enzymes. Some of these enzymes are involved in the production of H_2O_2 required by peroxidases. However, other enzymes catalyze the reduction of phenolic products derived from lignin degradation avoiding their posterior repolymerization. These enzymes include oxidases and reductases. Oxidases are grouped into: Glyoxal oxidase (GLOX; a copper radical enzyme) described in *P. chrysosporium* (Kersten and Kirk, 1987; Kersten, 1990; Takano et al., 2010), aryl alcohol oxidase (AAO) (or veratryl acohol oxidase, VAO) described in *P. eryngii* (Guillén et al., 1990; see Hernandez-Ortega et al., 2012), pyranose 2-oxidase or glucose 2-oxidase (Ruelius et al., 1968; Janssen and Ruelius, 1968a; Volc et al., 1978; Daniel et al., 1994), glucose oxidase (or glucose 1-oxidase) (Müller, 1928; Muller, 1936; Franke and Lorenz, 1937; Franke and Deffner, 1939; Kelley and Reddy, 1986), and alcohol oxidase (AOX) or methanol oxidase (Janssen et al., 1965; Janssen and Ruelius, 1968b; Suye, 1997). In addition, fungi produce reductases, such as aryl-alcohol dehydrogenases (AAD) (Muheim et al., 1991; Gutierrez et al., 1994), quinone reductases (QR) (Guillen et al., 1997; Bazzi, 2001) and CDH (or CBOR), CBO or CBQOR (Westermark and Eriksson, 1974a; Westermark and Eriksson, 1974b; Temp and Eggert, 1999).

- Pectinolytic Enzymes (Pectinases)

Pectinases are enzymes that catalyze the cleavage of pectic substances, such as pectin. Depending on the cleavage sites utilized, pectinases are categorized into one of three groups: esterases, lyases, and hydrolases (see Sharma et al., 2013b).

- Main Pectinases

The most studied pectinolytic enzymes are homogalacturonan-degrading enzymes: polygalacturonases (PG) or pectin depolymerase; polymethylgalacturonases (PMG); lyases or transeliminases and pectinesterases (PE), which is also known as pectin methylesterases (PME).

PGs catalyze the hydrolytic cleavage of α-1,4-glycosidic linkages in polygalacturonic acid chains by introducing water across the oxygen bridge to form D-galacturonate. They are classified into GH family 28 (Markovic and Janecek, 2001; Lombard et al., 2014; CAZy, 2015). They are divided into: endo-polygalacturonases (PGA) and exo-polygalacturonases (XPG). PGA randomly attacks the pectic acid (polygalacturonic acid) to produce a number of Gal A oligosaccharides. The enzyme cleaves internal α-1,4-D-glycosidic linkage between two low methyl esterified or non-methylated acid residues in pectic acid, because such enzyme can only act on glycosidic linkages adjacent to galacturonic acid residues with free carboxyl groups (Yuan and Boa, 1979; Mohamed et al., 2006). XPG catalyzes the hydrolysis of α-1,4-glycosidic linkages of homogalacturonan (HG) chains from the non-reducing end releasing monogalacturonate. The enzyme needs a non-esterified GalpA unit at subsites -2, -1 and +1 (Kester et al., 1999a). In addition, it is tolerant of xylose substitution by removal of the Gal A-Xyl dimer. As a consequence, XGA is also an XPG substrate (Beldman et al., 1996a; Kester et al., 1999b) (*see* figure 15a).

PMG performs the hydrolysis of α-1,4-glycosidic bonds of the pectin backbone and specially catalyzes highly esterified pectin, forming 6-methyl-D-galacturonate bonds (Seegmiller and Jansen, 1952) (*see* figure 15a).

Lyase performs the trans-eliminative reaction of the α-1,4 glycosidic bond of polygalacturonic acid polymer to form an Δ-4,5 unsaturated C-C bond at the non-reducing end of the cleaved pectin polysaccharide (Albersheim et al., 1960; Moran et al., 1968). They are classified as pectate lyase (pectate transeliminases or PGL) and pectin lyase, (pectin transeliminase or PL). PGL cleaves glycosidic linkages on pectin and produce unsaturated oligogalacturonates or digalacturonates. PGLs are usually specific for non-esterified pectin (pectate) and depend on Ca^{2+} (Starr and Moran, 1962; Pickersgill et al., 1994; Mayans et al., 1997; Seyedarabi et al., 2010). The enzyme is grouped into five of the polysaccharide lyase (POL) families 1, 2, 3, 9, and 10 (Lombard et al., 2014; CAZy, 2015). It exists two types of PGLs: endo-PGL, which acts on substrates at random internal sites within the chain, and exo-PGL, which catalyzes the substrate cleavage from the reducing end. PL catalyzes the random cleavage of high esterified pectin and produces unsaturated

methyloligogalacturonates. PL does not depend on Ca^{2+} (Albersheim et al., 1960; Edstrom and Phaff, 1963; Delgado et al., 1992; Vitali et al., 1998) (*see* figure 15c). PL belongs to POL family 1 (Lombard et al., 2014; CAZy, 2015).

PE or PME catalyzes the de-esterification of methyl ester linkages by removing the methoxyl group at O6 through catalyzing the hydrolysis of the ester linkage into pectate and methanol. The enzyme preferentially targets methyl ester groups in galacturonate units that are next to non-esterified galacturonate units (Solms and Deuel, 1955; Fries et al., 2007). The enzyme is active prior to PG and PGL enzymes, which require non-esterified substrates. After the action of PE, PGs and lyases, then PE catalyze the resulting pectin. PE is a part of CE family 8 (Lombard et al., 2014; CAZy, 2015) (*see* figure 15b).

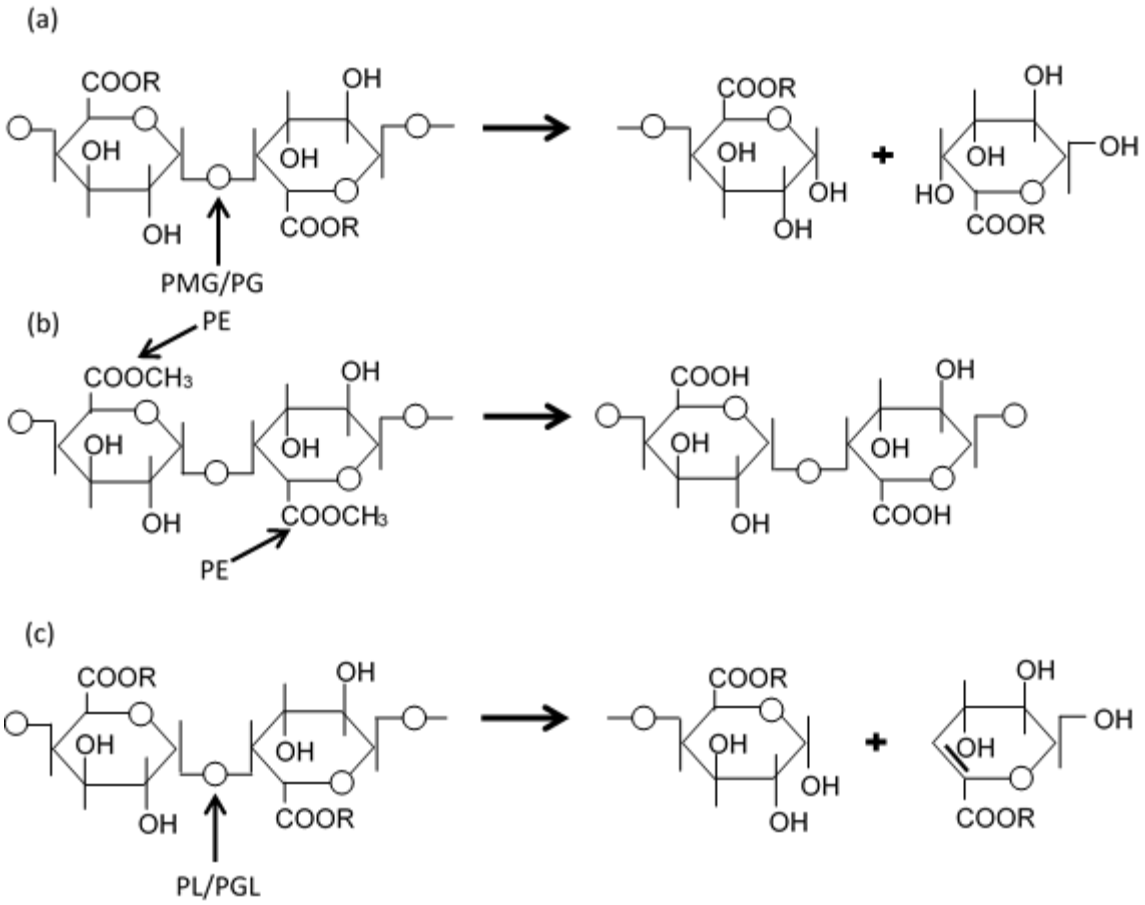

Figure 15. Mode of action of pectinases: (a) R = H for PG and CH3 for PMG; (b) PE; and (c) R = H for PGL and CH3 for PL. The place where the pectinolytic enzymes react with pectin are showed by the arrows. *Figure adopted from* (Pedrolli et al., 2009).

- Other Pectinases

Pectinases that have not been as extensively studied are: pectin acetyl esterases (PAE); rhammnogalacturonase (RGase), or also known as rhamnogalacturonan hydrolases (RGH); rhamnogalacturonan rhamnohydrolases (RGRH); rhamnogalacturonan galacturonohydrolases (RGGH); rhamnogalacturonan endolyases (RGL); rhamnogalacturonan acetylesterases (RGA); xylogalacturonan hydrolase (XGH); and accessory enzymes (*see* figure 16). They are either backbone degrading enzymes or debranching enzymes.

PAE hydrolyzes the acetyl ester group of HG and rhamnogalacturonan I (RG-I) forming pectic acid and acetate (Williamson et al., 1990; Williamson, 1991; Shevchik and Hugouvieux-Cotte-Pattat, 1997; Bolvig et al., 2003; Bonnin et al., 2008). PAE is classified into CE families 12 and 13 (Lombard et al., 2014; CAZy, 2015). RGase or RGH is an endo acting enzyme able to randomly hydrolyze the α-D-1,4-GalpA-α-L-1,2-Rhap linkage in the RG-I backbone producing oligogalacturonates. The enzyme is intolerant toward acetyl esterification of the RG-I backbone (Schols et al., 1990; Kofod et al., 1994). RGase is grouped into GH family 28 (Lombard et al., 2014; CAZy, 2015). RGRH is an exo-acting pectinase that catalyzes the hydrolytic cleavage of the rhamnogalacturonan chain of RG-I at the non-reducing end, which produces rhamnose (Mutter et al., 1994). This enzyme belongs to GH family 28 (Lombard et al., 2014; CAZy, 2015). RGGH is an exo-acting pectinase that catalyzes the hydrolytic cleavage of the rhamnogalacturonan chain of RG-I at the non-reducing end, which produces monogalacturonate (GalA moety) (Mutter et al., 1998a). RGGH is classified into the GH family 28 (Lombard et al., 2014; CAZy, 2015). RGL catalyzes the random transelimination (β-elimination) of the RG-I α-L-1,2-Rhap-α-D-1,4-GalpA backbone leaving an unsaturated galacturonate at the non-reducing end and a rhamnose at the reducing end (Kofod et al., 1994; Mutter et al., 1996). The RGL activity is hindered by the presence of the acetyl groups in the RG-I backbone (Kofod et al., 1994; Mutter et al., 1998b). These enzymes are classified as polysaccharide-lyase families 4 and 11 (Lombard et al., 2014; CAZy, 2015). RGA catalyzes the hydrolytic cleavage of acetyl groups from the rhamnogalacturonan chain in RG-I (Searle-van Leeuwen et al., 1992). RGA is classified into CE family 12 (Lombard et al., 2014; CAZy, 2015). XGH hydrolyzes the α-1,4-D linkages of xylose substituted galacturonan moieties in XGA producing xylose galacturonate dimers (van der Vlugt-Bergmans et al., 2000; Zandleven et al., 2005). XGH is grouped into GH family 28 (Lombard et al., 2014; CAZy, 2015). Accessory enzymes acting on the lateral chains of RG-I and rhamnogalacturonan II (RG-II) include endogalactanases, exogalactanases, AGLs and LACs, AFs, AR, exoarabinases and FAE (see de Vries and Visser, 2001).

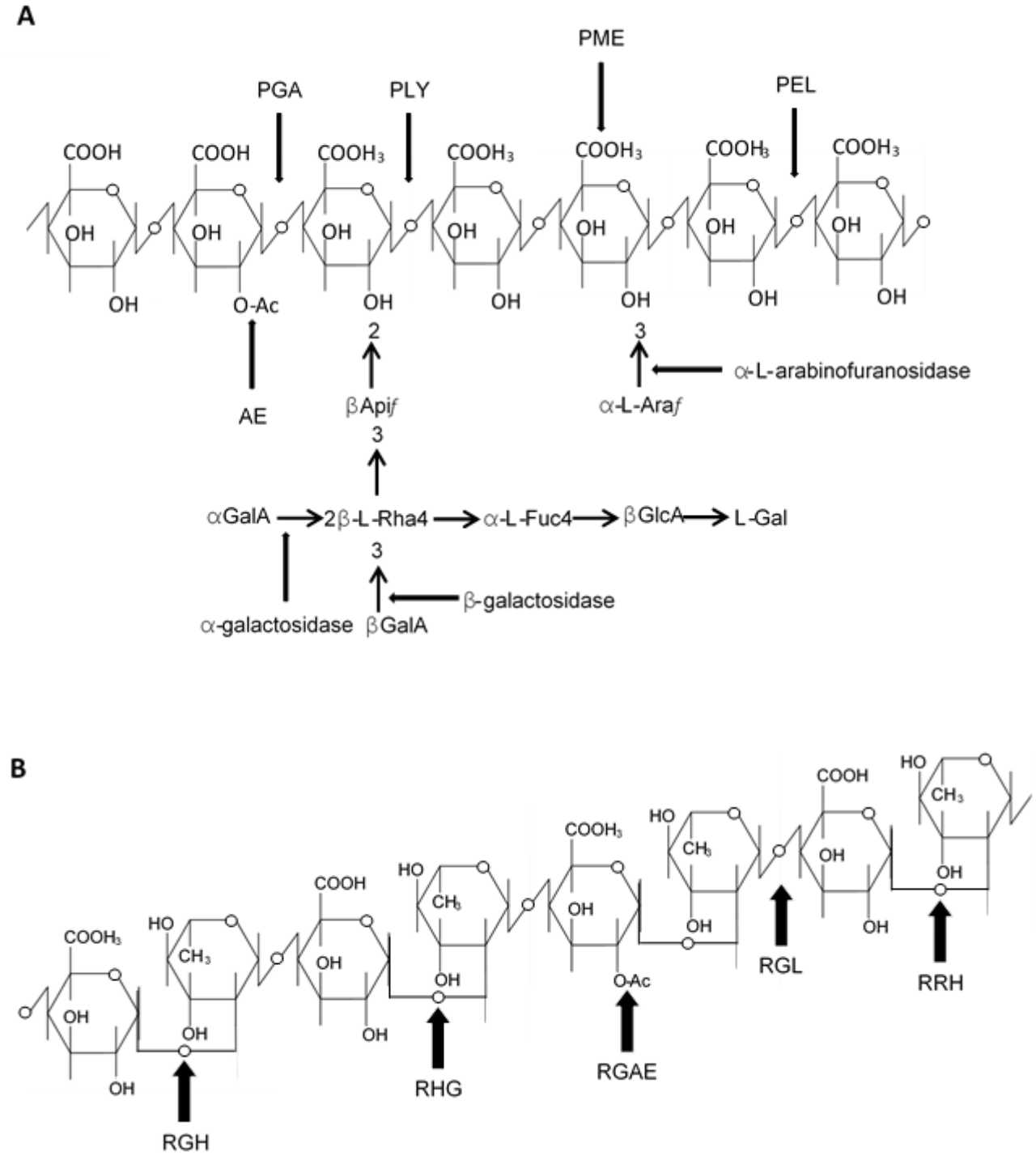

Figure 16. A-B schematic representation of HG and lateral chains of RG-II, and mode of action of pectinolytic enzymes involved in their degradation. *Figure adopted and modified from* (Lara-Márquez, Zavala-Páramo et al., 2011).

Natural Cellulosomes

Since bacteria and fungi are unable to engulf particles, these organisms need to secrete cellulases in order to degrade plant cell walls. The plant cell wall degrading apparatus of aerobic and anaerobic microorganisms differ considerably in their macromolecular organization. Cellulases and hemicellulases synthesized by anaerobes frequently assemble into a large multienzyme complex (molecular weight >2 MDa) called cellulosome (see Bégum and Lemaire, 1996; see Bayer et al., 1998; see Shoham et al., 1999; see Bayer et al., 2004; see Gilbert, 2007; see Smith and Bayer, 2013). Cellulases and hemicellulases produced by most aerobic microorganisms are free enzymes that are secreted at high concentrations and contain a CBM (Wilson, 2008).

Cellulosomes are supramolecular assemblies that are usually bound to the outer surface of the microorganism (see Smith and Bayer, 2013) and exploit the synergistic interactions of their enzyme components to efficiently degrade recalcitrant crystalline lignocellulosic substrates (Fierobe et al., 2002; Fierobe et al., 2005). A cellulosome from the anaerobic thermophilic bacterium *Clostridium thermocellum* was the first identified and characterized during the early 1980s (Bayer et al., 1983; Lamed et al., 1983a; Lamed et al., 1983b). The complex contains not only cellulases, but also a large array of hemicellulases (Morag et al., 1990; Kosugi et al., 2002) and pectinases (Tamaru and Doi, 2001). Enzyme activities include POLs, CEs, and GHs. Experimental evidence also demonstrates the presence of cellulosomes in anaerobic fungi that correspond to the genera Neocallimastix, Piromyces, and Orpinomyces (Wilson and Wood, 1992; Ali et al., 1995; Fanutti et al., 1995; Li et al., 1997; Fillingham et al., 1999; Steenbakkers et al., 2001; Steenbakkers et al., 2003; Nagy et al., 2007; Haitjema et al., 2013; Wang et al., 2014). In addition to the cellulosome, some anaerobic bacteria also produce free cellulases, but their function in cellulose degradation is still unknown (Gilad et al., 2003; Berger et al., 2007).

- Cellulosome Structure

The cellulosome is an extracellular protein complex on bacterial and fungal cell surfaces that adheres to plant materials and deconstructs plant cell wall lignocellulose (Lamed et al., 1983b). The cellulosome comprises several subunits, each of which displays a modular architecture. Some cellulosomes are structural and others are catalytic. The core structural components are known as scaffoldins, which attach all other subunits (Tokatlidis et al., 1991). The cellulosomal catalytic components contain non-catalytic modules called dockerins (Hall et al., 1988), which bind to the cohesin modules located in the scaffoldins (Tokatlidis et al., 1991; Schaeffer et al., 2002; Carvalho et al., 2003). The high affinity protein–protein interactions established between dockerins and

cohesins allow integration of the enzymes into the complex (Carvalho et al., 2003). In addition, scaffoldins usually contain a non-catalytic module called CBM that anchors the entire complex onto the plant cell wall (*see* figure 17).

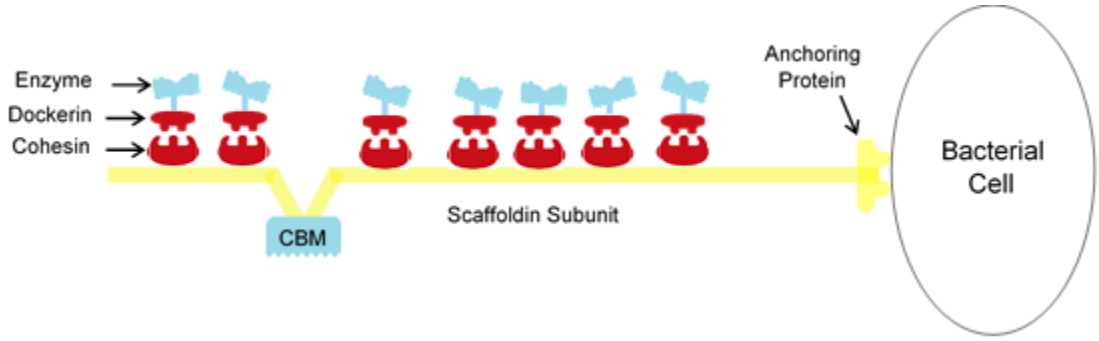

Figure 17. Basic schematic representation of the *C. thermocellum* cellulosome. *Figure adopted from* (Stahl et al., 2012).

Scaffoldins are large non-catalytic modular cohesin-containing proteins that are critical for cellulosome assembly and substrate binding through the CBM (Tokatlidis et al., 1991; Salamitou et al., 1992). Scaffoldins have been identified in most cellulosome-producing bacteria, such as *Acetivibrio cellulolyticus*, *Clostridium cellulolyticum*, *Clostridium cellulovorans* and *Clostridium josui* (Shoseyov et al., 1992; Kakiuchi et al., 1998; Pagès et al., 1999; Dassa et al., 2012). Dockerin contains a highly conserved duplicated segments of approximately 22 amino acids each connected by a peptide containing 8 to 17 amino acid residues and are usually present in a single copy at the C-terminus of cellulosomal enzymes (Grépinet and Béguin, 1986; Yagüe et al., 1990). The reaction between dockerins and cohesins requires Ca^{2+} in *C. thermocellum* (Yaron et al., 1995; Choi and Ljungdahl, 1996) and *C. cellulolyticum* (Pagès et al., 1997). The first 12 residues of each duplicated segment of dockerins resemble the calcium-binding loop in the EF-hand motif (Pagès et al., 1997). Ca^{2+} has shown to be essential for dockerin stability, function and compression into its tertiary structure (Choi and Ljungdahl, 1996; Lytle et al., 2000). These discoveries explain why Ca^{2+} is essential for the cohesin-dockerin interaction and hence the structural stability of the cellulosomes (Lytle et al., 1999; Lytle et al., 2000). The cohesin–dockerin interaction is among the highest affinity protein–protein interaction known. For example, the type I cohesin-dockerin interaction is characterized by a dissociation constant of the order of 10^{-10} M (Fierobe et al., 1999). The dockerin modules are believed to bind to cohesins in two different configurations. The dual binding mode would confer plasticity in dockerin-cohesin interactions and consequently, in cellulosome assembly, allowing the flexible incorporation of enzyme activities into the cellulosome. Furthermore, the plasticity in dockerin-cohesin recognition would also provide alternative modes of interaction between the enzymes and substrates (Carvalho et al., 2007). Cohesins are approximately 150-residue modules and are usually present as tandem repeats in

scaffoldins (Fujino et al., 1992; Shoseyov et al., 1992; Kakiuchi et al., 1998; Ding et al., 1999). There is a notable degree of amino acid homology between cohesins from different species. This similarity is also reflected in their structure; however, the cohesin-dockerin interactions appear to be species-specific (Pagès et al., 1997). The structure of the complex further revealed that protein-protein recognition is mainly mediated by hydrophobic interactions of the beta-sheet cohesin domain and one of the helices of the dockerin protein (Spinelli et al., 2000; Lytle et al., 2001; Miras et al., 2002; Schaeffer et al., 2002; Carvalho et al., 2003; Carvalho et al., 2007). In some cellulosome systems, including that of clostridial, the primary scaffoldin anchors the whole cellulosome onto the cell surface through interaction with a another type of cohesin from an anchoring protein (Leibovitz and Béguin, 1996). Most of the catalytic components of the cellulosome are devoid of the CBMs, and they depend on the CBM present on the scaffoldin protein for attachment to the polysaccharide substrates. Although, there are several reports that propose that CBMs disrupt crystalline structure (Knowles et al., 1987; Din et al., 1994; Wang et al., 2008), CBMs more likely act through targeting and proximity effect. They bring enzymes into intimate contact with plant cell walls enhancing proximity of the enzymes with it and thus, increasing the concentration of enzymes onto the polysaccharide substrates, which in turn enhances the catalytic efficiency of the associated enzymes (Black et al., 1996; Bolam et al., 1998; Hervé et al., 2010). They contain from 30 to approximately 200 amino acids and are located within the parental protein at either the C-terminal or N-terminal (Juge et al., 2002; Abe et al., 2004; Lunetta and Pappagianis, 2014; Peng et al., 2014). Originally, these domains were named, cellulose binding modules (CBD), because the first protein domains discovered bound primarily crystalline cellulose (Tilbeurgh et al., 1986; Gilkes et al., 1988; Tomme et al., 1988). Later, this name was replaced by CBM in order to reflect the diverse ligand specificity of these modules (Boraston et al., 1999). CBMs have been grouped into 67 families based on their amino acid sequences according to the CAZy database (Lombard et al., 2014; CAZy, 2015). Nevertheless, CBMs have also been classified into three types by Boraston based on their binding specificity (see Boraston et al., 2004). Type A CBMs interact with flat surfaces of insoluble polysaccharides, including crystalline cellulose; type B CBMs bind to internal regions of single polysaccharides (glycan chains); and type C CBMs recognize small saccharides such as mono-, di-, or trisaccharide. The orientation and positioning of the aromatic residues in the binding sites of CBMs are the primary drivers of specificity and affinity in these proteins (Simpson et al., 2000), but other interactions, including direct hydrogen bonds (Notenboom et al., 2001; Xie et al., 2001; Pell et al., 2003) and calcium-mediated coordination (Bolam et al., 2004; Jamal-Talabani et al., 2004), also play an important role in CBM ligand recognition. Some CBMs have become primordial to the substrate specificity and mode of action in cognate enzymes. For instance, family 3c CBMs may play a role in the processivity displayed by GH9 family 'endo-processive' cellulases (Sakon et al., 1997; Irwin et al., 1998; Li et al., 2007a; Burstein et al., 2009; Oliveira et al., 2009). Also, CBM 22 was proved to change the specificity of a GH10 family xylanase such that it displayed primarily β-1,4-β-1,3-glucanase activity (Araki et al., 2004).

- Biological Functions of Cellulosomes

It has been suggested that cellulosomes are more efficient at deconstructing plant structural polysaccharides than the corresponding "free" enzyme systems produced by aerobic bacteria and fungi. As an example, *C. thermocellum* requires much less protein in order to solubilize crystalline cellulose substrate than *T. reesei* (Johnson et al., 1982). Indeed, *C. thermocellum* holds one of the highest rates of cellulose hydrolysis (see Lynd et al., 2002). The *C. thermocellum* cellulosome displays a specific activity against crystalline cellulose, which is 50-fold higher than the corresponding *Tricoderma*-free cellulolytic system (see Demain et al., 2005). It has been reported that holding plant cell wall–degrading enzymes onto a macromolecular complex leads to a spatial enzyme proximity that maximizes the potential for synergy between different cellulosomal enzymes against recalcitrant substrates. These enzymes are further augmented by enzyme-substrate targeting scaffoldin-borne CBM (Fierobe et al., 2002; Fierobe et al., 2005). Several studies have shown the importance of recruiting lignocellulolytic enzymes into the cellulosome to mediate efficient hydrolysis of the crystalline substrate. This organization prevents non-productive adsorption of break down products by optimizing component spacing. Sets of enzymes with strong binding domains bind to a single site on the substrate, which prevents competition for a limited number of binding sites on the substrate. The presence of other enzymes with different specificities prevents hydrolytic cessation of one structural type of cellulose. Furthermore, it takes advantage of the synergistic interaction among the enzymes by the correct ratio between the components.

- Cellulosome of *Clostridium thermocellum*

C. thermocellum is a thermophilic and strictly anaerobic spore-forming bacterium, which hydrolyzes a wide range of polysaccharides from lignocellulosic biomass. More than thirty years ago, the *C. thermocellum* cellulosome was discovered. It was the first cellulosome discovered in a microorganism and is now one of the most widely studied nanomachines (Bayer et al., 1983; Lamed et al., 1983a; Lamed et al., 1983b; see Gilbert, 2007; see Bayer et al., 2008; see Fontes and Gilbert, 2010; see Kothari et al., 2011; see Akinosho et al., 2014). Crystalline cellulose is most efficiently degraded by this cellulosome (see Lynd et al., 2002). The extracellular enzyme complex has been shown to be > 2 MDa (Coughlan et al., 1985). In some strains, the cellulosomes aggregate with larger supercomplexes called polycellulosomes. Polycellulosomes have a molecular mass up to 100 MDa (Mayer et al., 1987). The complex composition varies with the carbon source (Bhat et al., 1993). Although *C. thermocellum* exclusively breaks down cellulose, the literature indicates that it has the potential to degrade a number of other polysaccharides (Spinnler et al., 1986; Zverlov et al., 2002b; Zverlov et al., 2005b).

- Structure

Several cellulosome-producing microbes express more than one type of scaffoldin. The primary scaffoldin of *C. thermocellum* is known as CipA (Lamed et al., 1983a; Gerngross et al., 1993; Kruus et al., 1995). CipA contains, nine type I cohesins that recognize type I dockerins in catalytic subunits, and a CBMIIIa, which binds crystalline cellulose and exhibits broad binding specificity for different sites on cell wall (Tokatlidis et al., 1991; Gerngross et al., 1993; Tormo et al., 1996; Blake et al., 2006; Yaniv et al., 2013). In addition, it contains a C-terminal type II dockerin, which is linked to a hydrophilic X-domain and does not recognize CipA cohesins. Instead, it recognizes type II cohesins located at the amino-terminal ends of cell-surface proteins, also known as anchoring proteins (SdbA, Orf2P, and OlpB), leading to the anchorage of cellulosomes or free enzymes to the cell (Lemaire et al., 1995; Leibovitz and Béguin, 1996; Leibovitz et al., 1997; Adams et al., 2006; Xu and Smith, 2010) (*see* figure 18).

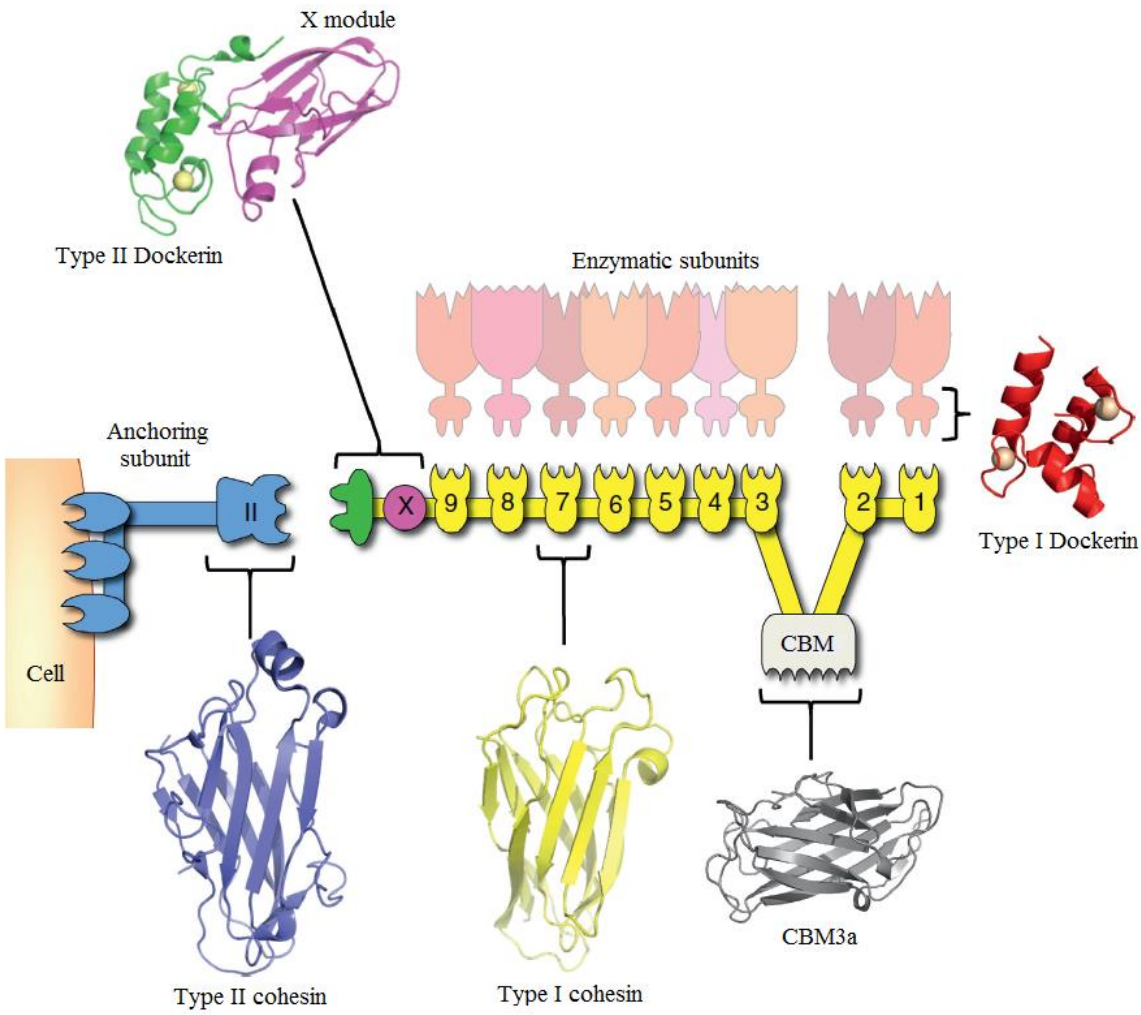

Figure 18. The schematic representation of the cellulosome from *Clostridium thermocellum* with the X-ray crystal structures of the individual cellulosomal components. *Figure adopted from* (Smith and Bayer, 2013).

As it was mentioned earlier, cohesin-dockerin interactions appear to be species-specific (Pagès et al., 1997). However, there are exceptions, and thus, the Xyn11A dockerin of *C. thermocellum* binds to various cohesins of *C. josui* with high affinities (KD of 10^{-8} M) (Jindou et al., 2004). Cohesin I domains are connected to the scaffoldin by O-glycosylated linker segments of about 20 residues containing a majority of proline, serine, and threonine (Gerwig et al., 1989; Gerwig et al., 1991; Gerwig et al., 1992; Gerwig et al., 1993). Inter-cohesin linkers appear intrinsically disordered and display a high degree of conformational flexibility (Hammel et al., 2004; Hammel et al., 2005; Bomble et al., 2011; Garcia-Alvarez et al., 2011; Currie et al., 2012; Currie et al., 2013). The interaction of *C. thermocellum* type-I cohesion-dockerin complex structure is achieved via a binding interface with an extensive hydrogen-bonding network and supporting hydrophobic interactions. These interactions primarily involve only one of the two main helices present in the

symmetric dockerin structure and a face of the cohesin module formed by strands 8, 3, 6, and 5 (Carvalho et al., 2003). Structural and mutagenesis data reveal that type I dockerins contain two almost identical cohesin binding interfaces. Residues participating in cohesin recognition at the two binding interfaces (specifically a serine-threonine pair at positions 11 and 12 and a lysine-arginine pair at positions 18 and 19) are highly conserved in the two segments of the majority of *C. thermocellum* dockerins. This suggests that both binding interfaces display similar protein specificities (Carvalho et al., 2003; Karpol et al., 2008; Garcia-Alvarez et al., 2011). The plasticity of the type-I cohesion-dockerin interaction may reduce steric constraints, which allows the enzymes to assume an alternative conformation for substrate degradation (Carvalho et al., 2007). It also facilitates dockerin alternation through recognition of unbound cohesins, which leads to a continuous reorganization of the cellulosome. This provides the structural flexibility necessary to enhance substrate targeting and improves the synergistic interactions between additional enzymes, including exo- and endo-acting cellulases (Carvalho et al., 2003; Carvalho et al., 2007). Most significantly, type I and type II cohesin/dockerin partners do not interact. This ensures that there is a clear distinction between the mechanism for cellulosome assembly and cell-surface attachment (Leibovitz and Béguin, 1996). The CBMIIIa from CipA corresponds to family 3 of CBM. The crystal structure of CBM3 from *C. thermocellum* displays a nine-stranded β-sandwich fold and one β-sheet presenting a planar topology, which interacts with crystalline cellulose. It is an internal domain that consists of approximately 155 residues (Tormo et al., 1996; Yaniv et al., 2013). In comparison to other type A CBMs, CBM3s bind more extensively to cellulose (Blake et al., 2006). The absorption of the CBM to cellulose depends on the structure arrangement of the cellulosic substrate. The CBM from *Clostridium thermocellum* possesses higher binding capacity for amorphous cellulose than for crystalline cellulose. The capacity for amorphous cellulose is 20 fold higher (Morag et al., 1995). The X module is involved in the dockerin stability and cohesion recognition (Adams et al., 2006). The CipA dockerin II module binds only to the type II cohesins of one cell wall binding protein or anchoring scaffolding SdbA, Orf2P, or OlpB (which carry 1, 2 and 4 type II cohesin(s), respectively) in a highly specific and ultra-tight manner (Leibovitz et al., 1997; Adams et al., 2006). In addition, two type-II cohesin-containing CipA anchoring proteins (Cthe_0735 and Cthe_0736) have been found. The latter comprises 7 type-II cohesin modules, which increases the potential to form polycellulosome structures (Raman et al., 2009; Raman et al., 2011). Two type-I cohesin-containing cell-surface anchoring proteins (OlpA and OlpC) have also been identified, which bind individual cellulosome components (Salamitou et al., 1994; Pinheiro et al., 2009) (see figure19).

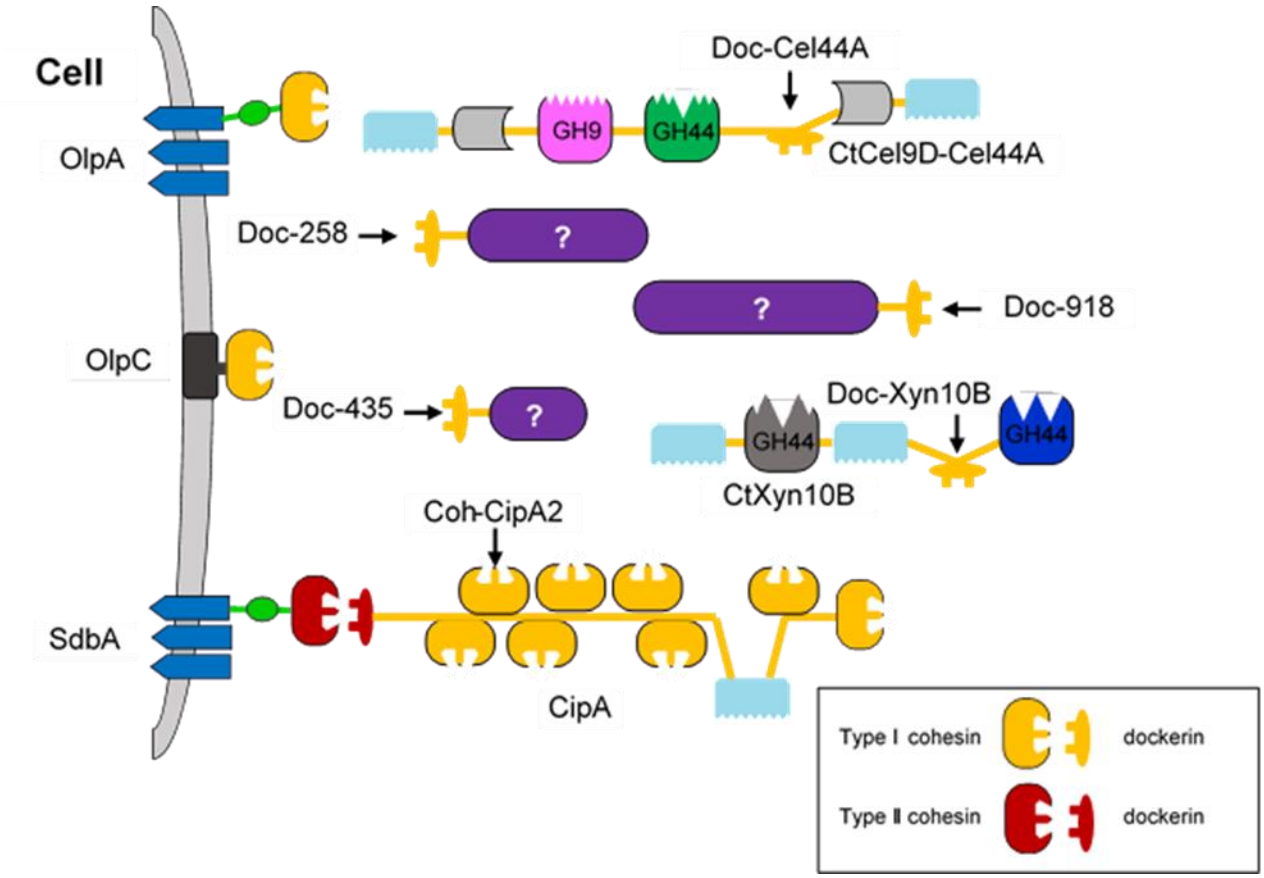

Figure 19. Simplified representation of *C. thermocellum cellulosome* with cell-anchored proteins, OlpA and OlpC and SdbA. *Figure adopted from* (Pinheiro et al., 2009).

While SdbA, OlpB, and Orf2 in *C. thermocellum* are bound to the peptidoglycan layer of the cell envelope, OlpA and OlpC interact with secondary cell wall polymers in the S-layer of the cell envelope (Lemaire et al., 1995; Zhao et al., 2006a; Zhao et al., 2006b; Pinheiro et al., 2009). However, the biological significance of these identified differences in cell-wall specificity remains unclear.

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- Catalytic Subunits

Several cellulosomal enzymes have been revealed in *Clostridium thermocellum*. More specifically, the complex is comprised of numerous EGs (Shinmyo et al., 1979; Ait et al., 1979a; Garcia-Martinez et al., 1980; Ng and Zeikus, 1981; Petre et al., 1981; Beguin et al., 1983; Cornet et al., 1983; Béguin et al., 1985; Pétré et al., 1986; Schwarz et al., 1986; Joliff et al., 1986a; Joliff et al., 1986b; Soutschek-Bauer and Staudenbauer, 1987; Schwarz et al., 1988; Mel'nik et al., 1989;

Hazlewood et al., 1990; Fauth et al., 1991; Jung et al., 1992; Romaniec et al., 1992; Kobayashi et al., 1993; Mosolova et al., 1993; Singh and Akimenko, 1994; Bhat et al., 2001; Zverlov et al., 2003; Zverlov et al., 2005a); four EXs or CBHs, which include CbhA (formerly Cbh3; component S3) (Tuka et al., 1990; Mel'nik et al., 1991; Singh and Akimenko, 1993; Zverlov et al., 1998) CelS (S8) (Wang et al., 1993; Wang and Wu, 1993), CelK (S5) (Kataeva et al., 1999), and CelO (Zverlov et al., 2002a); a CBP (Sih and McBee, 1955; Alexander, 1968); a CDP (Sheth and Alexander, 1967; Sheth and Alexander, 1969); two βGs (bglA and bglB) (Aït et al., 1979b; Gräbnitz et al., 1989; Gräbnitz et al., 1991); at least six ENs (XynA, XynB, XynC, XynY, XynZ and XynD) (Grépinet et al., 1988; Morag et al., 1990; Fontes et al., 1995; Hayashi et al., 1997; Hayashi et al., 1999; Zverlov et al., 2005b), which comprise xylan esterase modules in XynY and XynZ to remove feruloyl residues from native xylan (Blum et al., 2000); one XGH (XghA) (Zverlov et al., 2005b); two lichenases (1,3-1,4-β-glucanases) (Schwarz et al., 1985; Schimming et al., 1991); two laminarinases (1,3-β-glucanases) (Tuka et al., 1990); and minor activities of βX, LAC, and MND (Kohring et al., 1990). *C. thermocellum* has been shown to degrade pectin and probably produces pectin lyase, polygalacturonate hydrolase, pectin methylesterase (Spinnler et al., 1986), one chitinase (Chi18A) (Zverlov et al., 2002b), and one MAN (Halstead et al., 1999).

- Lignocellulolytic System of *Tricorderma reesei*

Most aerobic microorganisms, including fungi (e.g. *Trichoderma reesei*), the bacterium *Thermobifida fusca*, and other aerobic bacteria do not possess a cellulosome for the degradation of lignocellulolytic material. Instead, they produce single enzyme components at high concentrations, which are connected to synergistic binding modules (Wilson, 2008). These enzymes also play an important role in degrading the polysaccharide component of biomass, and are mostly from fungi that belong to the genus *Trichoderma* (Gosh and Gosh, 1992) in particular, *Trichoderma reesei.* *Trichoderma reesei* (anamorph of *Hypocrea jecorina*) is a filamentous mesophilic soft-rot ascomycete fungus that is widely used in industry as a source for cellulases and hemicellulases in the hydrolysis of plant cell wall polysaccharides (Kuhls et al., 1996; Merino and Cherry, 2007). The exceptional capacity for extracellular protein production and the ability to grow on a wide range of substrates make filamentous fungi the source of choice for industrial enzymes (Schaffner and Toledo, 1991). *T. reesei* is particularly efficient at producing extracellular enzymes. Certain industrial strains produce over 100 grams of extracellular proteins per liter (see Schuster and Schmoll, 2010). *T. reesei* serves as the primary industrial source for cellulases and hemicellulases (Merino and Cherry, 2007), which are useful in pulp and paper industries (Buchert et al., 1998), textile industries (Galante et al., 1998), food and feed industries (Hjortkjaer et al., 1986; Roldán et al., 2009), and biofuel production (Kataria and Ghosh, 2011). The production of the primary *T. reesei* enzymes that are related to lignocellulosic biomass degradation is transcriptionally regulated, and the relative proportions of expressed proteins may vary widely

depending on the growth medium used, carbon source, and cultivation conditions (Allen and Roche, 1989; Foreman et al., 2003; Juhasz et al., 2005; Stricker et al., 2008; Sipos et al., 2010; Maurya et al., 2012; Coffman et al., 2014). For example, the production of ENs and βX by *T. reesei* is induced very specifically by lactose and xylose, respectively (Kristufek et al., 1995; Xiong et al., 2004). The genome sequence of *T. reesei* has helped researchers identify the diversity of hydrolytic enzymes secreted by this fungus (Martinez et al., 2008). Unexpectedly, previous studies have indicated that *T. reesei* produces a relatively smaller number of cellulases, hemicellulases, and pectinases compared to other plant cell wall degrading fungi (Martinez et al., 2008). Enzyme expression by *T. ressei* depends on the carbon sources (Jun et al., 2013b). Protein engineering have been used in these enzymes to improve properties, such as alkali-tolerance, stability or activity (Wang et al., 2005a; Nakazawa et al., 2009).

- Catalytic Subunits

T. reesei has the smallest repertoire of genes for cellulases, hemicellulases, and pectinases (Martinez et al., 2008). As a result, several enzyme families involved in polysaccharide degradation are reduced or absent in *T. reesei.*

Carbohydrate Active Enzymes (CAZymes) are enzymes that degrade, modify, or create glycosidic bonds, which include GHs (enzymes that hydrolyze or rearrange glycosidic bonds), glycosyltransferases (GTs; enzymes that form glycosidic bonds), POLs (enzymes with a non-hydrolytic cleavage mechanism for glycosidic bonds) and CEs (enzymes that hydrolyse carbohydrate esters). Many of the *T. reesei* genes encoding CAZymes involved in polysaccharide degradation are not distributed randomly in the genome; instead, they are located in clusters. In general, the *T. reesei* genome encodes a number of CAZymes that are slightly below the average found among Sordariomycetes (Martinez et al., 2008). Unexpectedly, the number of genes encoding GHs (201) is below average for the number of GHs found in Sordariomycetes (Martinez et al., 2008; Hakkinen et al., 2012). The *T. reesei* genome also has the smallest number of CBM-containing proteins (36) among the Sordariomycetes (Martinez et al., 2008). In addition, *T. reesei* contains 22 CEs and 5 POL genes (Hakkinen et al., 2012). With respect to the content of GTs (99), however, *T. reesei* is close to average within the same lineage (Martinez et al., 2008; Hakkinen et al., 2012). *T. reesei* lacks several protein families that are important for lignocellulosic degradation. These enzymes are included in PGL, pectin esterase, tannase, and FAE families. Of all possible CAZyme genes involved in pectin degradation, T. reesei possesses the smallest amount of pectinolytic enzymes (family GH28) among the plant cell wall–degrading fungi. The enzyme invertase is also absent (family GH32) (Martinez et al., 2008).

The components of the *T. reesei* cellulolytic system include: two CBHs (CBHI/CEL6A and CBHII/CEL7A) (Shoemaker et al., 1983; Teeri et al., 1983; Teeri et al., 1987); ten EGs, which include five characterized enzymes (EGI/CEL7B, EGII/CEL5A, EGIII/CEL12A, EGIV/CEL61A and EGV/CEL45A) (Penttilä et al., 1986; Saloheimo et al., 1988; Saloheimo et al., 1994; Saloheimo et al., 1997; Okada et al., 1998) and five putative enzymes (CEL5B, CEL61B, CEL74A, gene ID 53731, gene ID 77284) (Foreman et al., 2003; Martinez et al., 2008; The Regents of the University of California, 2015); and eleven βGs, which comprise two characterized enzymes (BGLI/CEL3A, BGLII/CEL1A) (Barnett et al., 1991; Fowler and Brown, 1992; Takashima et al., 1999; Saloheimo et al., 2002) and nine candidate enzymes (CEL3B, CEL3D, CEL1B, CEL3C, CEL3E, bgl3i, gene ID 66832, bgl3j and bgl3f) (Foreman et al., 2003; Ouyang et al., 2006; Martinez et al., 2008). Both CBHs have been shown to act processively. Whereas CEL6A cleaves the cellobiose dimers from the non-reducing end of the cellulose chain, CEL7A acts from the reducing end (Barr et al., 1996). The GH5 cellulase CEL5B has a putative GPI (Glycophosphatidylinositol)-anchor at the C-terminus, which binds this protein to the plasma membrane and fungal cell wall. CEL74A was later characterized as a putative XGH (Grishutin et al., 2004). Enzymes from the GH family 61 have been shown to enhance lignocellulosic degradation by an oxidative mechanism (Langston et al., 2011). In addition, several novel candidate cellulolytic enzymes have been identified from the genome of *T. reesei* (Foreman et al., 2003).

There are several hemicellulases produced by *T. reesei*: seven ENs, that include four characterized ENs from the families GH10 (XYNIII), GH11 (XYNI, XYNII) and GH30 (XYNIV) (Tenkanen et al., 1992; Torronen et al., 1992; Xu et al., 1998), and three candidates enzymes (gene ID 112392 or XYNV, 41248 and 69276) (Metz et al., 2011; The Regents of the University of California, 2015); one MAN (MANI) (Stålbrand et al., 1995); one candidate AXL (gene ID: 69944); one candidate β-1,3-mannanase (or gen ID 71554); six candidate MNDs (gene ID: 5836, 69245, 59689, 57857, 62166 and 71554) (The Regents of the University of California, 2015); four AXEs, which comprise one characterized enzymes (AXEI) and three predicted enzymes (AXEII and gene ID: 70021 and 54219) (Margolles-Clark et al., 1996a; Foreman et al., 2003; Herpoel-Gimbert et al., 2008; The Regents of the University of California, 2015); a candidate cutinase (gene ID60489) (The Regents of the University of California, 2015); a putative XGH (CEL74A) (Grishutin et al., 2004); two AgluAs, one characterized from the family GH67 (GLRI) (Margolles-Clark et al., 1996b) and a candidate from the family GH115 (gene ID 79606) (Hakkinen et al., 2012); five AFs, that include one characterized AF (ABFI) (Margolles-Clark et al., 1996c) and four candidate AFs (ABFII,ABFIII and gene ID 3739 and 68064) (Foreman et al., 2003; Herpoel-Gimbert et al., 2008; The Regents of the University of California, 2015); nine AGLs, that consist of three characterized enzymes (AGLI, AGLII, AGLIII) (Zeilinger et al., 1993; Margolles-Clark et al., 1996d) and six candidate enzymes (gen ID: 27219, 27259, 59391, 75015, 55999 and 65986) (Metz et al., 2011; The Regents of the University of California, 2015); two LACs, which comprise one characterized

enzymes (bga1) and a candidate enzyme (gene ID: 76852) (Seiboth et al., 2005; The Regents of the University of California, 2015); five βXs, that consist of one characterized enzyme (BXLI) (Margolles-Clark et al., 1996c) and four candidates (xyl3b and gen IDs: 73102, 3739 and 68064) (Ouyang et al., 2006; The Regents of the University of California, 2015); two AXEs, one characterized (AESI) (Li et al., 2008b) and one candidate (gene ID: 103825) (Hakkinen et al., 2012); one characterized GE (CIPII) (Foreman et al., 2003; Li et al., 2007b; Pokkuluri et al., 2011); five candidate β-glucuronidases (gene ID: 76852, 71394, 106575, and 73005) (The Regents of the University of California, 2015); two α-glucuronidases, one characterized (GLRI) (Margolles-Clark et al., 1996b) and one candidate enzyme (gen ID: 79606) (Hakkinen et al., 2012); and five candidate AFUs (gene ID: 69944, 72488, 5807, 111138 and 58802) (The Regents of the University of California, 2015).

Engineered Enzymes

Natural lignocellulolytic enzymes have limitations when used in industrial processes. When compared to chemical catalysis, biocatalysis provides enormous advantages, including high efficiency, high degree of selectivity (regio-, chemo-, and enantio-), and ‘‘green’’ reaction conditions (see Hudlicky and Reed, 2009; see Reetz, 2009). Thus, it is not strange that industrial catalysis becomes more and more dependent on enzymes. However, most naturally occurring enzymes are not optimized for industrial applications. Multiple traits need to be satisfied to create the ideal industrial enzyme catalyst (see Burton et al., 2002). The search for superior enzymes has been the interest of many researchers over the past decade. In order to overcome the limitations of naturally occurring enzymes, researchers have developed different methods to obtain biocatalysts with better traits. Over the years these methods have changed. For many years, the identification of better biocatalysts depended only on labor-intensive screening of microbial cultures to achieve the desired activities. The basis for this process was that isolated cultures permit extended and reproducible growth, which in turn allow phenotypic and genotypic characterization (Ferrés et al., 2015). But only a small number of microbes can successfully be cultivated in the laboratory. Thus, this approach will miss the majority of the biodiversity found in nature. Another strategy is the metagenome approach, which allows the sequence of entire genomes from environmental samples by the extraction of genomic DNA, fragmentation, and clonation to yield the corresponding metagenome libraries (see Handelsman et al., 1998; Srivastava et al., 2013). This process allows faster access to catalytic activities from organisms that cannot be cultured, but screening larger libraries of DNA or microbes may not be the fastest or most efficient route to obtain a good catalyst. One of the latest and most promising alternatives to address this problem is protein engineering, which uses molecular biological methods and/or computational techniques to adapt enzyme functions for applied ends. There are two general

approaches for protein engineering: rational design and directed evolution. Although there is a third approach for protein improvement based on statistical analysis, it is used less often.

In the case of lignocellulosic biofuels, the high cost of enzymes is a major obstacle for economic and industrial production of cellulosic biofuels. The high cost is attributable to the large amounts of cellulases that are required to breakdown cellulose into fermentable sugars (Merino and Cherry, 2007; Klein-Marcuschamer et al., 2012). A cost effective enzyme technology used to degrade polysaccharides into fermentable sugars is crucial for economically viable biofuels. In order to address these issues, four primary strategies can be carried out: (1) metagenome screening (see Handelsman et al., 1998; Srivastava et al., 2013); (2) genome mining in sequenced microbial genomes (see Ahmed, 2009; see Davidsen et al., 2010); (3) exploring the diversity of extremophiles (see Schiraldi and De Rosa, 2002; see Kumar et al., 2011b); and (4) developing superior enzymes, such as cellulases with improved characteristics, including higher catalytic efficiencies, increased stability at elevated temperatures, and higher tolerance to end product inhibition, using advanced biotechnology like enzyme engineering, which plays an important role in developing superior enzymes including cellulases. Currently, protein engineering is a well-established technology for modifying the properties of enzymes; general strategies, as well as numerous successful examples, have been published (Peters et al., 2003; seeKazlauskas and Bornscheuer, 2009; see Turner, 2009; see Bornscheuer et al., 2012; see Davids et al., 2013). Two enzymatic engineering strategies have been proposed for lignocellulolytic enzymes: (1) improving properties of individual cellulases; (2) synergy engineering by optimizing the enzyme cocktails for maximized synergy or by the creation of a multi-enzyme cellulolytic complex called cellulosome (Zhou et al., 2009; see Mohanram et al., 2013; Ji et al., 2014; Hu et al., 2015). So far, the most remarkable results in protein engineering of cellulases are improvements in thermostability and thus, diverse thermostable cellulases have been constructed (Heinzelman et al., 2009a; Heinzelman et al., 2009b; Heinzelman et al., 2010; Komor et al., 2012; Smith et al., 2012; Wu and Arnold, 2013; Trudeau et al., 2014). On the other hand, commercial development of hemicellulases for enzymatic hydrolysis of lignocellulosic material is not as advanced as cellulases, since current commercial enzymes mixtures have mostly been developed for the hydrolysis of biomass pretreated with acid, which removes the majority of hemicellulose (Pedersen et al., 2011).

- Engineered Individual Enzymes

Engineering for single enzymes is achieved via rational design, which is primarily based on the enzymes structure knowledge and the catalytic mechanism (Johnsson et al., 1993; Pleiss, 2012); or directed evolution, in which the improved enzymes or ones with new properties, are selected or

screened after random mutagenesis, molecular recombination or focused mutagenesis (see Packer and Liu, 2015) (see figure 20).

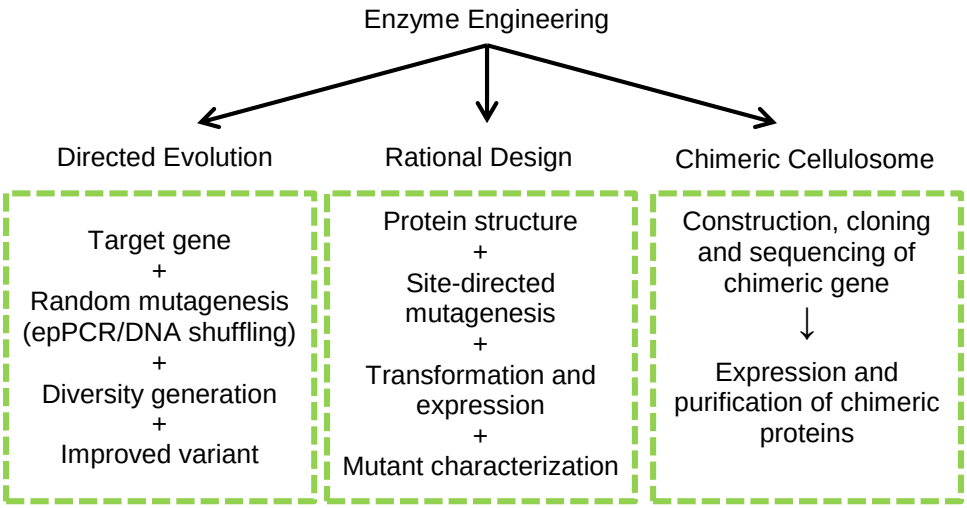

Figure 20. Routes to advancement in cellulase enzyme technology. *Figure adopted and modified from* (Mohanram et al., 2013).

- Rational Design

Ratinal design is the oldest technique in protein engineering introduced after the invention of recombinant DNA methods and site-directed mutagenesis. Rational design uses biochemical data, protein structures and molecular modeling data to propose mutations that would be introduced by site-specific mutagenesis. This approach is dependent on enzyme structural information, function and mechanism. The process of rational design involves: (1) choice of a suitable enzyme, (2) identification of the amino acid sites to be changed, typically based on a high resolution crystallographic structure, and (3) characterization of the mutants via sequencing and purification of the mutant enzymes following each round of mutagenesis (Johnsson et al., 1993; Pleiss, 2012). Assuming that structural information on the target enzyme is available, rational design may be the easiest and fastest approach to enzyme engineering. Computational models (or in silico studies) are typically used to predict which amino acid(s) should be altered in the protein (see Tiwari et al., 2012). Then, targeted mutagenesis is used to change the corresponding gene. Rational design requires the knowledge of the enzymatic structure of interest and/or its sequence in several related species. As a result, crystallography and spectroscopic analysis has been a powerful tool for computer modeling. Advances in modeling, specifically calculations of free energy perturbation and molecular dynamics, help predict mutations. Techniques that help identify mutations include

algorithms (see Desjarlais and Clarke, 1998). Thermostability improvement using rational design via molecular dynamic simulations is applied without reducing enzyme activity. This occurs by taking into account protein-surface properties instead of protein-core characteristics, such as core packing and cavity filling (Joo et al., 2010; Joo et al., 2011). Similarly, the integration of different structural prediction techniques can be applied in the rational design of enzymes. This is also the case for molecular docking, fragment molecular orbital (FMO) calculation, and three-dimensional quantitative structure-activity relationship with comparative molecular field analysis (3D-QSAR CoMFA) modeling (Zhang et al., 2008). Moreover, the creation of structural databases helps researchers understand the functional role of individual amino acids within the enzymatic structure. Among the huge number of data analysis software, two new internet-based computational tools are particularly useful: (1) The HotSpot Wizard server and (2) the commercial 3DM database. The HotSpot Wizard server combines information from extensive sequence and structural database searches with functional data to create a mutability map for a target protein (Pavelka et al., 2009). Likewise, the commercial 3DM database integrates protein sequence and structural data from GenBank and the PDB to create comprehensive alignments of protein super families (Kuipers et al., 2010). A new strategy involves the use of nuclear magnetic resonance relaxation (NMR) dispersion experiments coupled with mutagenesis studies for enzyme catalysis. NMR methods provide a powerful tool to help characterize the effects of controlling long range networks of flexible residues that affect enzymatic function (see Doucet, 2011). Another new approach involves the combination of site direct mutagenesis with immobilization. This approach offers support to improve the activity, stability, and selectivity of the immobilization of biocatalysts (see Mateo et al., 2007; see Hernandez and Fernandez-Lafuente, 2011). However, the application of pure rational design is still limited by some factors, such as the lack of understanding of structural properties and their contributions to function, or the limited knowledge of protein dynamics (Ruscio et al., 2009).

Site-directed mutagenesis on cellulases was first reported using the *T. reesei* exo I gene (Chen et al., 1987). The goal of this experiment was to provide information on the role of individual residues during catalysis. Recently, thermostability of βGs from *Trichoderma reesei* and *Penicillium piceum* H16 have been improved by rational design (Lee et al., 2012; Zong et al., 2015). Also, Arnold and coworkers used a computational approach and site-directed mutagenesis to produce a thermostable fungal CBHI I (Cel7A) and achieved a 10°C increase in optimal active temperature (Komor et al., 2012). Another study reported the shifting of the pH optimum of an EG (PvEGIII) from *Penicillium verruculosum* using a rational design approach (Tishkov et al., 2013). Escovar-Kousan and his team reported an increase of 40% in the activity of the *T. fusca* EG/EX Cel9A on amorphous cellulose or soluble cellulose using the same method (Escovar-Kousen et al., 2004). In addition, Rignall and coworkers changed significantly the mixtures of products released from phosphoric acid swollen cellulose by a single mutation in the active site cleft of the EG-I from *Acidothermus cellulolyticus* (Rignall et al., 2002). On the other hand, to

date only a few cellulases modified by site directed mutagenesis have been reported to possess significantly higher activity on insoluble substrates. One significant example used a modified EG Cel5A from Acidothermus cellulolyticus to report a 20% improvement in its activity against microcrystalline cellulose by decreasing product inhibition (Baker et al., 2005). In some cases, mutant enzymes with higher activity do not increase the activity of a synergistic mixture containing several cellulases (Zhang et al., 2000). Although the research on cellulase mechanisms started by 1950, the mechanism by which cellulases catalyze the hydrolysis of crystalline cellulose is not entirely understood. There is insufficient data regarding the mechanism by which a cellulase binds a segment of a cellulose chain from a microfibril into its active site. Also, there is an incomplete understanding of the way in which certain free CBM stimulate cellulase hydrolysis (Moser et al., 2008; Wang et al., 2008). Finally, although the mechanism for cellulase synergism has been studied and documented (see Woodward, 1991; see Kostylev and Wilson, 2012), there is still much more to understand about this essential process: in particular, how mixtures of cellulases hydrolyze both crystalline and amorphous regions of bacterial cellulose, while most individual enzymes only seem to degrade amorphous regions (Chen et al., 2007). Therefore, rational design is limited, since it requires a detailed understanding of structure–function relationships for cellulase crystalline cellulose activity, which is still lacking. Other enzymes that have also been engineered by rational design are hemicellulases. In this case, rational design has been used in order to shift the pH optimal (Pokhrel et al., 2013; Xu et al., 2013a; Xu et al., 2013b) or improve the thermostability (Fonseca-Maldonado et al., 2013; Satyanarayana, 2013) or the catalytic performance (Huang et al., 2014; Cheng et al., 2015). On the other hand, although some studies can be found regarding the application of rational design on ligninolytic enzymes and pectinases in other to improve their properties, much less research has been published (Xiao et al., 2008; Fang et al., 2014). Rational design has also been used to engineer methabolic pathways (Eriksen et al., 2014).

- Directed Evolution

Directed evolution is another choice for engineering individual enzymes. It has become the most important tool for improving critical traits of biocatalysts, including the improvement of thermal (Koksharov and Ugarova, 2011; Steffler et al., 2013; Zhou et al., 2015), oxidative or activity stability (Stemmer, 1994a; Oh et al., 2002; Akbulut et al., 2013; Wang et al., 2015), enantio-selectivity (Reetz et al., 1997; May et al., 2000; Kim et al., 2015b), pH range (Ness et al., 1999; Wang et al., 2005a; Melzer et al., 2015), substrate specificity (Glieder et al., 2002; Gupta and Farinas, 2010; Ng et al., 2015) and tolerance or stability towards organic solvents (Moore and Arnold, 1996; Reetz et al., 2010a; Yamada et al., 2015). As rational design, directed evolution can also be applied to engineer metabolic pathways and even whole organisms (see Eriksen et al., 2014; see Guenther et al., 2014). Furthermore, directed evolution can be applied to the generation of individual novel enzyme functions (Raillard et al., 2001; Chen and Zhao, 2005). Applying this

approach to the genomes of whole organisms provides the potential to evolve whole-cell biocatalysts within a whole sequence process (Patnaik et al., 2002; Snoek et al., 2015). Directed evolution tends to be more successful than the rational approach (see Gerlt and Babbitt, 2009). With directed evolution, which requires only knowledge of the protein sequence, the amino acid sequence of an enzyme is iteratively altered until the enzyme functions in the desired manner. The process involves iterative cycles of producing mutants and finding the mutant with the desired properties via screening or selection (see Arnold, 1998) (*see* figure 21).

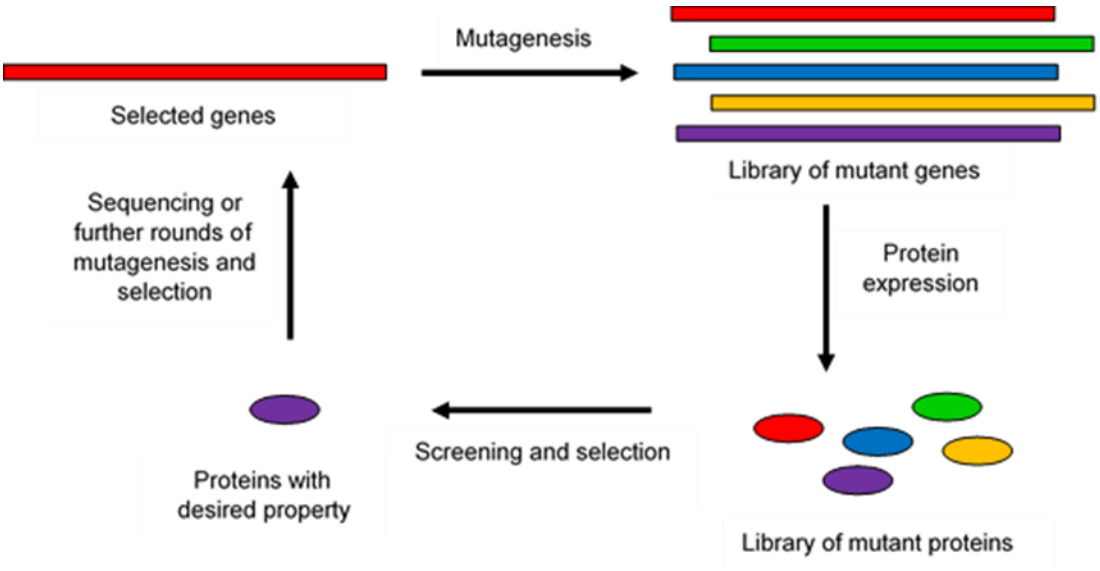

Figure 21. General steps of directed enzyme evolution. The gene encoding the protein of interest is mutated to generate a library of mutant genes. Expression of the mutant genes provides the library of mutant proteins. The proteins are screened or selected based on a desired property, and the variants with modified activity are sequenced or used for further rounds of mutagenesis and selection. *Figure adopted from* (Tao and Cornish, 2002).

The idea of directed evolution for biomolecules in vitro and on a molecular level was first introduced by the pioneering work of Spiegleman et al. (Mills et al., 1967). Regarding nucleic acids, Eigen and Kauffman (Kauffman, 1993) proposed a theory for molecular evolution. Arnold's group was among the first to apply the principles of molecular evolution for the creation of improved enzymatic biocatalysts. They improved the activity of the protease subtilisin E in organic co-solvents (Chen and Arnold, 1993). The greatest advantage of directed evolution compared to rational design is that it is independent of the knowledge of enzyme structure and the interactions between enzyme and substrate. This allows scientists to engineer enzymes whose functions are not yet fully understood (*see* figure 22).

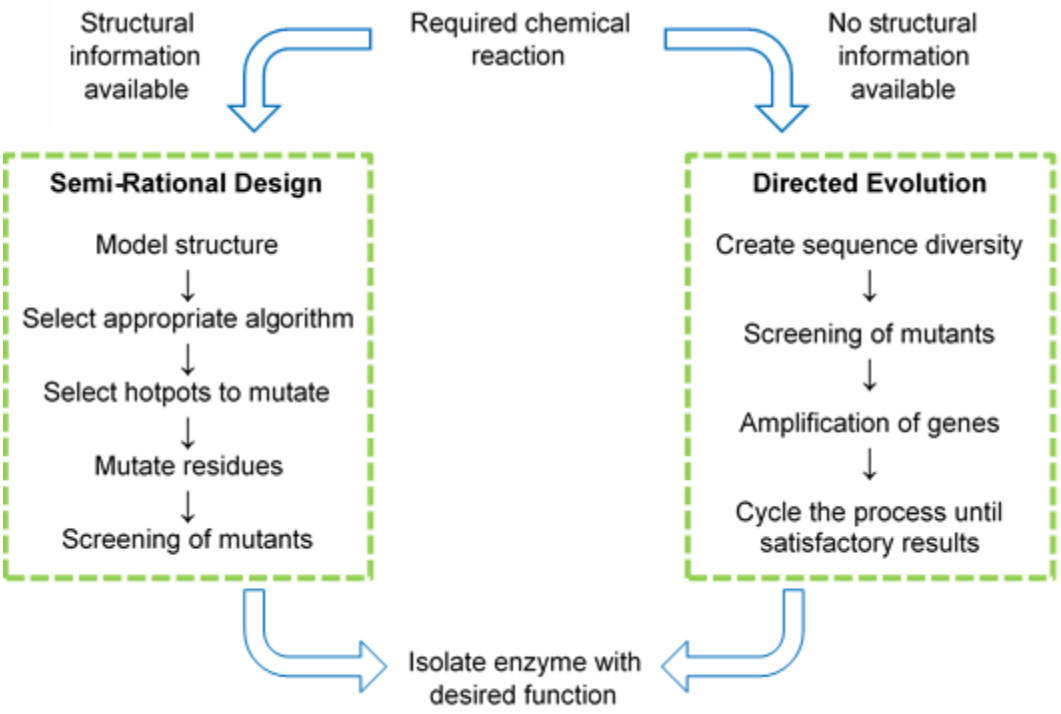

Figure 22. Summary of the different processes required by semirational design and directed evolution. *Figure adopted from* (Quin and Schmidt-Dannert, 2011).

In general, mutations can be introduced throughout the gene via three strategies: (1) random mutagenesis, (2) random recombination or (3) focused mutagenesis (see Packer and Liu, 2015). A wide variety of methods have been developed to create a mutant library. The most commonly employed techniques are: error-prone PCR (epPCR), which was first described by Goeddel and coworkers (Leung et al., 1989), and DNA shuffling , which was first recognized by Pim Stemmer (Stemmer, 1994a; Stemmer, 1994b). The epPCR inserts random point mutations across genes due to the low fidelity the Taq polymerase under certain conditions, such as the increase of magnesium concentrations, supplementation with manganese, or the use of mutagenic dNTP analogues (Zaccolo et al., 1996). The DNA shuffling method is based on mixing and subsequent joining of different, but related small DNA fragments, forming a complete new gene. It is typically achieved by creating hybrid gene libraries via the homologous recombination of related parent genes (Crameri et al., 1998). Non-homologous recombination can also be employed (Sieber et al., 2001). New directed evolutionary methods have been used, including Look-Through Mutagenesis (LTM), which was developed as a method for rapid screening of amino acid mutations in protein sequence selected positions that introduce favorable properties, and Combinatorial Beneficial Mutagenesis (CBM), which is used to identify the best ensemble of individual mutations (Hokanson et al., 2011). The success of a directed evolutionary experiment depends highly on the method that is used to find the best mutant enzyme among a large number of mutants in the library

(You and Arnold, 1996). Identifying interesting variants within large combinatorial libraries (generated by most directed evolution experiments) can be accomplished either by assaying all the members individually (screening) or applying conditions that allow only variants of interest to appear (selection) (see Packer and Liu, 2015). In general, selective methods are preferred over screening methods, because of the higher performance (see Olsen et al., 2000; Griffiths et al., 2004; see Otten and Quax, 2005; Seelig, 2011). The advantage of screening is that the difference between substrate and product of an enzymatic reaction can be determined directly or indirectly in almost every case. The disadvantage of screening is that all individual mutants have to be tested in order for the desired enzymatic reaction to occur. This even includes mutants that might not be active, accurately folded, or when there is a possibility of a high percentage of mutants. Furthermore, the development of a high-throughput screening method is not available for all enzyme stabilities and not all screening methods are easy to implement at the required scale. Screening can be performed via facilitated screening, which distinguishes mutants on the basis of distinct phenotypes, including chromospheres released and halos formed. If facilitated screening is not available, then random screening is utilized, which randomly chooses mutants (see Taylor et al., 2001). The last screening method is often implemented using microtiter plates along with fluorescent substances (Yang et al., 2010; Despotovic et al., 2012; Yu et al., 2014; Zeng et al., 2015). It can also be achieved utilizing chromatography, mass spectrometry, capillary electrophoresis, or IR-thermography (see Wahler and Reymond, 2001). Selection mimics the survival of the fittest strategy. The primary advantage of selection over screening is that many more library members can be analyzed simultaneously, because uninteresting variants are not observed. Consequently, library surveying is much faster and can be carried out with higher output. In the best screening protocols available, the maximum number of library members that can be assayed is approximately 10^8. In contrast, up to 10^{13} clones can be assessed with selection methods (see Packer and Liu, 2015). Selection is based on the advantage that mutants with the desired enzyme function provide to the host cell over bacteria bearing wild type enzymes. Although, many enzymatic activities are not essential to the bacterium, by coupling the activity of interest to an essential feature of the bacterium, this can be changed. Selection approach is often carried out based on the principles of resistance to cytotoxic agents (e.g., antibiotics) (Stemmer, 1994a; Stemmer, 1994b; Siau et al., 2015) or complementation with an auxotroph (Smiley and Benkovic, 1994; Jürgens et al., 2000; Griffiths et al., 2004; De Groeve et al., 2009). Despite the fact that directed evolution is a powerful method to overcome some of the limitations of biocatalysts, it possesses some limitations. Evolutionary analysis of enzymatic families suggest that drastic changes in enzyme function might require significant changes in polypeptide backbones (Matsumura, 2000). However, with in vitro evolution, this cannot be achieved since enzymes are improved only by point mutations, which have a significant bias for transitions over transversions. This limits access to a broader spectrum of substitutions. Another limitation is the considerable time required to implement, because the number of all possible protein variants (including the inactive ones) in a directed evolution experiment is too large. Thus, more recently, the emphasis has been on producing smaller libraries (although also including diversity) and higher

quality libraries. In order to reduce the large number of mutants in the library, one approach is to employ a more efficient screening. Modifications in substrate specificity may be monitored with high-output methods, such as fluorescence-activated cell sorting (Bernath et al., 2004; Becker et al., 2008; Fernandez-Alvaro et al., 2011), which can screen tens of millions of variants in a short amount of time. Directed evolution is also sometimes employed in conjunction with rational design to produce 'smarter libraries' (semi-rational approach) (Ba et al., 2013; Teze et al., 2015; Zhang et al., 2015). The prior knowledge of either the sequence or the three-dimensional crystal structure of the enzyme leads to the design of a more specific set of mutations, which allows creation of a much smaller library with a higher proportion of mutants displaying beneficial traits. This approach takes advantage of rational and random protein design to produce smaller smarter libraries and make the directed evolution faster and more efficient (Reetz et al., 2010b). Another strategy to reduce the number of mutants in libraries involves limiting the location of changes in the active site. The type of changes to those known from sequence comparisons often occur at these sites and mutations closer to these regions seem to be more beneficial (Morley and Kazlauskas, 2005; Jochens and Bornscheuer, 2010; Liebgott et al., 2010). In addition, an important advance that allows multiple mutations is the recognition that mutations often destabilize proteins (Guo et al., 2004; Drummond et al., 2005; see Tokuriki and Tawfik, 2009; Worth et al., 2011). Starting with a very stable protein allows it to tolerate a greater number and range of changes (Bloom et al., 2007; Gupta and Tawfik, 2008). Two more approaches involve the assumption that beneficial mutations are mostly additive (Wells, 1990) and that synergistic effects are rare with the exception of nearby changes. Subsequently, the number of useful changes made during the improvement of a protein has increased considerably in the past decade and the size of the libraries has decreased. The number of mutations has increased from 1–5 mutations in the early 2000s, to 30–40 amino-acid substitutions by 2010 (Fox et al., 2007; Savile et al., 2010).

Some properties of several enzymes involved in lignocellulosic degradation have been changed using directed evolutionary techniques. They are mostly EGLs (Kim et al., 2000; Murashima et al., 2002b; Catcheside et al., 2003; Wang et al., 2005a; Nakazawa et al., 2009; Liu et al., 2010; Liang et al., 2011; Vu and Kim, 2012; Liu et al., 2013a; Lehmann et al., 2014) and βGs (Arrizubieta and Polaina, 2000; González-Blasco et al., 2000; Lebbink et al., 2000; McCarthy et al., 2004; Hardiman et al., 2010; Pei et al., 2011; Drevland et al., 2014) whose activities were principally assayed in a high throughput manner with the help of artificial substrates, either soluble or chromogenic. Only a few examples of directed evolution on EXs existed due to a lack of reliable screening methods (Wang et al., 2012f; Wu and Arnold, 2013). Directed evolution has only achieved moderate success on improving individual cellulases. This is primarily due to the difficulties in developing high throughput screening methods on activities towards the insoluble cellulosic substrates (see Zhang et al., 2006b). Most of the cellulases that have been obtained by directed evolution were screened on artificial substrates and the enzymes have not shown considerable improvement in the hydrolysis of natural substrates (Lin et al., 2009; Nakazawa et

al., 2009; Hardiman et al., 2010). As a result, high throughput screening on natural substrates is needed, although it is difficult to carry out (see Zhang et al., 2006b; Liu et al., 2010). Recently, researchers tried to address these challenges during the development of various automated microplate platforms, which can evaluate the enzymatic hydrolysis of lignocellulose in a high throughput manner (Chundawat et al., 2008; Navarro et al., 2010; Song et al., 2010; Bharadwaj et al., 2011). These automated high throughput systems would greatly facilitate future protein engineering on biomass degrading enzymes or enzyme cocktails. Directed evolution has also been used to change properties of hemicellulases, such as pH optimum (Ruller et al., 2014), thermostability, (Singh et al., 2014; Zheng et al., 2014) or activity (Wang et al., 2013; Du et al., 2014). In addition, directed evolution has been used for the improvement of ligninolytic enzymes and pectinases however, not as much work has been published about it (Solbak et al., 2005; Garcia-Ruiz et al., 2012; Liu et al., 2013b; Viña-Gonzalez et al., 2015; Zhou et al., 2015).

CONCLUSION

The aim of this paper has been to review both seminal and current research and technologies regarding the use and development of enzyme systems for deconstructing lignocellulosic materials into simpler sugars, which can then be fermented to generate bioethanol products. Descriptions of natural enzymes and explanations of the modes and action of engineered systems illustrate the complexity and challenges in efficiently reducing cellulose such that bioethanol may be produced in cost-competitive manner.

The composition and structural organization of cellulolytic enzyme systems from two classes of microorganisms indicate that lignocellulose degradation is efficiently achieved in nature. However, attempts to artificially mimic natural systems in whole or in part have proven difficult. Indeed, the literature demonstrates large variations in efficiency and particularities in the modes of action of different plausible enzyme-based solutions to lignocellulose deconstruction. Nonetheless, advances in the selection of synergistic enzyme complements, the engineering of highly catalytic cellulolytic enzymes, and the development of enzyme sequestration platforms provide promising technological avenues to overcome current challenges.

Future breakthroughs in engineered enzyme systems may soon boost process efficiency so that cost-competitive commercial-scale bioethanol may be produced. Such breakthroughs may include: engineering enzymes based on the improvement of select structural-functional features; synergy engineering for enzyme cocktails; and/or the development of enzyme sequestration

platforms that increase enzymatic efficiency. Enzymes can be engineered by rational design, based on knowledge of the enzyme's structure and catalytic mechanism or by directed evolution. Given that the structure and catalytic mechanisms for many enzymes are not available, only a few studies using rational design have been successful in making significant improvements in overall sugar reduction efficiency. Directed evolution, which possesses the advantage of only requiring the knowledge of the protein sequence, is a powerful emerging technology that may result in novel solutions to the lignocellulose deconstruction problem. However, two limitations must be addressed in directed evolution. First, only point mutations can currently be performed by this method, which limits the magnitude of change that can be induced with respect to enzyme function. If significant change in an enzyme's sequence is required before a high performing enzyme evolves, then the single point mutation approach may be prohibitively time consuming. Furthermore, due to the large number of possible protein sequence variants, a method to narrow down libraries will be required. Perhaps, a hybrid approach that combines both rational design and directed evolution or a "semi-rational" approach will ultimately prove to be the best method for generating high performance cellulolytic enzymes. This can lead to the development of a more specific set of variants. Indeed, recent work on EGs and βGs support this possibility. However, advanced high throughput screening methods for assessing enzymatic activity will need to be developed before semi-rational design is touted as the best option for development of cellulolytic enzymes.

In conclusion, the development of processes for enhancing the production of ethanol from natural feedstocks has substantially progressed over the past few decades; however, further development of enzyme systems and industrial scale testing of technologies is required before an environment-friendly hazardous materials-free solution is achieved.

Abe, A., T. Tonozuka, Y. Sakano and S. Kamitori (2004). "Complex structures of Thermoactinomyces vulgaris R-47 alpha-amylase 1 with malto-oligosaccharides demonstrate the role of domain N acting as a starch-binding domain." J Mol Biol. **335**(3): 811-822.
Adams, J. J., G. Pal, Z. Jia and S. P. Smith (2006). "Mechanism of bacterial cell-surface attachment revealed by the structure of cellulosomal type II cohesin-dockerin complex." Proc Natl Acad Sci U S A **103**(2): 305-310.
Adav, S. S., C. S. Ng, M. Arulmani and S. K. Sze (2010). "Quantitative iTRAQ secretome analysis of cellulolytic Thermobifida fusca." J Proteome Res. **9**(6): 3016-3024.
Ademark, P., R. P. de Vries, P. Hagglund, H. Stalbrand and J. Visser (2001). "Cloning and characterization of Aspergillus niger genes encoding an alpha-galactosidase and a beta-mannosidase involved in galactomannan degradation." Eur J Biochem **268**(10): 2982-2890.
Ahmad, M., J. N. Roberts, E. M. Hardiman, R. Singh, L. D. Eltis and T. D. Bugg (2011). "Identification of DypB from Rhodococcus jostii RHA1 as a lignin peroxidase." Biochemistry **50**: 5096-5107.
Ahmed, N. (2009). "A flood of microbial genomes-do we need more?" PLoS One **4**(6): e5831.
Ahmed, S., A. S. Luis, J. L. Bras, A. Ghosh, S. Gautam, M. N. Gupta, C. M. Fontes and A. Goyal (2013). "A novel α-L-arabinofuranosidase of family 43 glycoside hydrolase (Ct43Araf) from Clostridium thermocellum." PloS one **8**(9): e73575.
Aït, N., N. Creuzet and J. Cattanéo (1979b). "Characterization and purification of thermostable β-glucosidase from Clostridium thermocellum." Biochemical and Biophysical Research Communications **90**(2): 537-546.
Ait, N., N. Creuzet and P. Forget (1979a). "Partial purification of cellulase from Clostridium thermocellum." Journal of General Microbiology **113**(2): 399-402.
Akbulut, N., M. T. Öztürk, T. Pijning, S. İ. Öztürk and F. Gümüşel (2013). "Improved activity and thermostability of Bacillus pumilus lipase by directed evolution." J Biotechnol. **164**(1): 123-129.
Akinosho, H., K. Yee, D. Close and A. Ragauskas (2014). "The emergence of Clostridium thermocellum as a high utility candidate for consolidated bioprocessing applications." Front Chem. **2**(66).
Albersheim, P., H. Neukom and H. Deuel (1960). "Über die Bildung von ungesättigten Abbauprodukten durch ein pektinabbauendes Enzym." Helv. Chim. Acta **43**(5): 1422-1426.
Alexander, J. K. (1961). "Characteristics of cellobiose phosphorylase." J Bacteriol **81**: 903-910.
Alexander, J. K. (1968). "Purification and specificity of cellobiose phosphorylase from Clostridium thermocellum." Journal of Biological Chemistry **243**(11): 2899-2904.
Ali, B. R. S., L. Zhou, F. M. Graves, R. B. Freedman, G. W. Black, H. J. Gilbert and G. P. Hazlewood (1995). "Cellulases and hemicellulases of the anaerobic fungus Piromyces constitute a multiprotein cellulose-binding complex and are encoded by multigene families." FEMS Microbiology Letters **125**(1): 15-22.
Allen, A. L. and C. D. Roche (1989). "Effects of strain and fermentation conditions on production of cellulase by Trichoderma reesei." Biotechnology and Bioengineering **33**(5): 650-656.
Ander, P. and K.-E. Eriksson (1985). "Methanol formation during lignin degradation by Phanerochaete chrysosporium." Applied Microbiology and Biotechnology **21**(1-2): 96-102.
Ander, P., K.-E. Eriksson and H.-s. Yu (1983). "Vanillic acid metabolism by Sporotrichum pulverulentum: evidence for demethoxylation before ring-cleavage." Archives of Microbiology **136**(1): 1-6.

Andreotti, G., A. Giordano, A. Tramice, E. Mollo and A. Trincone (2005). "Purification and characterization of a β-D-mannosidase from the marine anaspidean Aplysia fasciata." J Biotechnol. **119**(1): 26-35.
Andrić, P., A. S. Meyer, P. A. Jensen and K. Dam-Johansen (2010). "Reactor design for minimizing product inhibition during enzymatic lignocellulose hydrolysis: I. Significance and mechanism of cellobiose and glucose inhibition on cellulolytic enzymes." Biotechnol. Adv. **28**(3): 308-324.
Aracri, E. and T. Vidal (2011). "Xylanase- and laccase-aided hexenuronic acids and lignin removal from specialty sisal fibres." Carbohydrate Polymers **83**: 1355-1362.
Arai, M., R. akamoto and S. Murao (1989). "Different action by two Avicelases from Aspergillus aculeatus." Agric. BioI. Chern. **53**(5): 1411-1412.
Araki, R., M. K. Ali, M. Sakka, T. Kimura, K. Sakka and K. Ohmiya (2004). "Essential role of the family-22 carbohydrate-binding modules for beta-1,3-1,4-glucanase activity of Clostridium stercorarium Xyn10B." FEBS Lett **561**(1-3): 155-158.
Ariza, A., J. M. Eklöf, O. Spadiut, W. A. Offen, S. M. Roberts, W. Besenmatter, E. P. Friis, M. Skjøt, K. S. Wilson, H. Brumer and G. Davies (2011). "Structure and activity of Paenibacillus polymyxa xyloglucanase from glycoside hydrolase family 44." Journal of Biological Chemistry **286**(39).
Armand, S., S. Drouillard, M. Schulein, B. Henrissat and H. Driguez (1997). "A bifunctionalized fluorogenic tetrasaccharide as a substrate to study cellulases." J Biol Chem **272**(5): 2709-2713.
Arnold, F. H. (1998). "Design by directed evolution." Accounts of Chemical Research **31**(3): 125-131.
Arora, D. S. and R. K. Sharma (2009). "Enhancement in in vitro digestibility of wheat straw obtained from different geographical regions during solid state fermentation by white rot fungi." Bioresources **4**(3): 909-920.
Arrizubieta, M. a. J. and J. Polaina (2000). "Increased thermal resistance and modification of the catalytic properties of a β-glucosidase by random mutagenesis andin vitro recombination." Journal of Biological Chemistry **275**(37): 28843-28848.
Ayers, A. R., S. B. Ayers and K. E. Eriksson (1978). "Cellobiose oxidase, purification and partial characterization of a hemoprotein from Sporotrichum pulverulentum." Eur J Biochem. **90**(1): 171-181.
Ayers, W. A. (1959). "Phosphorolysis and synthesis of cellobiose by cell extracts from Ruminococcus flavefaciens." Journal of Biological Chemistry **234**(11): 2819-2822.
Ba, L., P. Li, H. Zhang, Y. Duan and Z. Lin (2013). "Semi-rational engineering of cytochrome P450sca-2 in a hybrid system for enhanced catalytic activity: insights into the important role of electron transfer." Biotechnol Bioeng. **110**(11): 2815-2825.
Baker, J. O., J. R. McCarley, R. Lovett, C. H. Yu, W. S. Adney, T. R. Rignall, T. B. Vinzant, S. R. Decker, J. Sakon and M. E. Himmel (2005). "Catalytically enhanced endocellulase Cel5A from Acidothermus cellulolyticus." Appl Biochem Biotechnol **121-124**: 129-148.
Baldrian, P. (2006). "Fungal laccases - occurrence and properties." FEMS Microbiol Rev **30**(2): 215-242.
Barnett, C. C., R. M. Berka and T. Fowler (1991). "Cloning and amplification of the gene encoding an extracellular β-glucosidase from Trichoderma reesei: Evidence for improved rates of saccharification of cellulosic substrates." Nat Biotech **9**(6): 562-567.

Barr, B., Y. Hsieh, B. Ganem and D. Wilson (1996). "Identification of two functionally different classes of exocellulases." Biochemistry **35**(2): 586 - 592.
Barr, B. K., Y. L. Hsieh, B. Ganem and D. B. Wilson (1996). "Identification of two functionally different classes of exocellulases." Biochemistry **35**(2): 586-592.
Barras, D. R., A. E. Moore and B. A. Stone (1969). "Enzyme-substrate relations among β-glucan hydrolases." Adv. Chem. Ser. **95**: 105-138.
Barreca, A. M., M. Fabbrini, C. Galli, P. Gentili and S. Ljunggren (2003). "Laccase/mediated oxidation of a lignin model for improved delignification procedures." Journal of Molecular Catalysis B: Enzymatic **26**(1-2): 105-110.
Bayer, E. A., J.-P. Belaich, Y. Shoham and R. Lamed (2004). "The cellulosome: multienzyme machines for degradation of plant cell wall polysaccharides." Annual Review of Microbiology **58**(1): 521-554.
Bayer, E. A., H. Chanzy, R. Lamed and Y. Shoham (1998). "Cellulose, cellulases and cellulosomes." Current Opinion in Structural Biology **8**(5): 548-557.
Bayer, E. A., R. Kenig and R. Lamed (1983). "Adherence of Clostridium thermocellum to cellulose." J Bacteriol **156**(2): 818-827.
Bayer, E. A., R. Lamed, B. A. White and H. J. Flint (2008). "From cellulosomes to cellulosomics." The Chemical Record **8**(6): 364-377.
Bazzi, M. D. (2001). "Interaction of camel lens zeta-crystallin with quinones: portrait of a substrate by fluorescence spectroscopy." Arch. Biochem. Biophys. **395**(2): 185-190.
Becker, S., H. Hobenreich, A. Vogel, J. Knorr, S. Wilhelm, F. Rosenau, K. E. Jaeger, M. T. Reetz and H. Kolmar (2008). "Single-cell high-throughput screening to identify enantioselective hydrolytic enzymes." Angew Chem Int Ed Engl **47**(27): 5085-5088.
Beeson, W. T., A. T. Iavarone, C. D. Hausmann, J. H. Cate and M. A. Marletta (2011). "Extracellular aldonolactonase from Myceliophthora thermophila." Appl Environ Microbiol **77**(2): 650-656.
Beeson, W. T., C. M. Phillips, J. H. Cate and M. A. Marletta (2012). "Oxidative cleavage of cellulose by fungal copper-dependent polysaccharide monooxygenases." J Am Chem Soc **134**(2): 890-892.
Béguin, P., P. Cornet and J. P. Aubert (1985). "Sequence of a cellulase gene of the thermophilic bacterium Clostridium thermocellum." Journal of Bacteriology **162**(1): 102-105.
Beguin, P., P. Cornet and J. Millet (1983). "Identification of the endoglucanase encoded by the celB gene of Clostridium thermocellum." Biochimie **65**(8-9): 495-500.
Begum, M. F. and N. Absar (2009). "Purification and Characterization of Intracellular Cellulase from Aspergillus oryzae ITCC-4857.01." Mycobiology **37**(2): 121-127.
Bégum, P. and M. Lemaire (1996). "The cellulosome: an exocellular, multiprotein complex specialized in cellulose degradation." Critical Reviews in Biochemistry and Molecular Biology **31**(3): 201-236.
Beldman, G., L. A. M. van den Broek, H. A. Schols, M. J. F. Searle-van Leeuwen, K. M. J. van Laere and A. G. J. Voragen (1996a). "An exogalacturonase from Aspergillus aculeatus able to degrade xylogalacturonan." Biotechnology Letters **18**(6): 707-712.
Benoit, I., E. G. Danchin, R. J. Bleichrodt and R. P. de Vries (2008). "Biotechnological applications and potential of fungal feruloyl esterases based on prevalence, classification and biochemical diversity." Biotechnol Lett **30**(3): 387-396.

Bentley, R. and A. Neuberger (1949). "The mechanism of the action of Notatin." Biochem. J. **45**: 584-590.
Berger, E., D. Zhang, V. V. Zverlov and W. H. Schwarz (2007). "Two noncellulosomal cellulases of Clostridium thermocellum, Cel9I and Cel48Y, hydrolyse crystalline cellulose synergistically." FEMS Microbiol Lett **268**(2): 194-201.
Berghem, L. E. and L. G. Pettersson (1973). "The mechanism of enzymatic cellulose degradation. Purification of a cellulolytic enzyme from Trichoderma viride active on highly ordered cellulose." Eur J Biochem. **37**(1): 21-30.
Berghem, L. E., L. G. Pettersson and U. B. Axiö-Fredriksson (1976). "The mechanism of enzymatic cellulose degradation. Purification and some properties of two different 1,4beta-glucan glucanohydrolases from Trichoderma viride." Eur J Biochem. **61**(2): 621-630.
Berlin, A., N. Gilkes, D. Kilburn, R. Bura, A. Markov, A. Skomarovsky, O. Okunev, A. Gusakov, V. Maximenko, D. Gregg, A. Sinitsyn and J. Saddler (2005a). "Evaluation of novel fungal cellulase preparations for ability to hydrolyze softwood substrates – evidence for the role of accessory enzymes." Enzyme and Microbial Technology **37**(2): 175-184.
Bernath, K., M. Hai, E. Mastrobattista, A. D. Griffiths, S. Magdassi and D. S. Tawfik (2004). "In vitro compartmentalization by double emulsions: sorting and gene enrichment by fluorescence activated cell sorting." Anal Biochem **325**(1): 151-157.
Bharadwaj, R., A. Wong, B. Knierim, S. Singh, B. M. Holmes, M. Auer, B. A. Simmons, P. D. Adams and A. K. Singh (2011). "High-throughput enzymatic hydrolysis of lignocellulosic biomass via in-situ regeneration." Bioresour Technol. **102**(2): 1329-1337.
Bhat, S., P. W. Goodenough, E. Owen and M. K. Bhat (1993). "Cellobiose: A true inducer of cellulosome in different strains of Clostridium thermocellum." FEMS Microbiology Letters **111**(1): 73-78.
Bhat, S., E. Owen and M. K. Bhat (2001). "Isolation and Characterisation of a Major Cellobiohydrolase (S8) and a Major Endoglucanase (S11) Subunit from the Cellulosome of Clostridium thermocellum." Anaerobe **7**(3): 171-179.
Biely, P. (2012). "Microbial carbohydrate esterases deacetylating plant polysaccharides." Biotechnol Adv. **30**(6): 1575-1588
Biely, P. (2012). "Microbial carbohydrate esterases deacetylating plant polysaccharides." Biotechnology Advances **30**(6): 1575-1588.
Biely, P., M. Vrsanska, M. Tenkanen and D. Kluepfel (1997). "Endo-beta-1,4-xylanase families: differences in catalytic properties." J Biotechnol **57**(1-3): 151-166.
Black, G. W., J. E. Rixon, J. H. Clarke, G. P. Hazlewood, M. K. Theodorou, P. Morris and H. J. Gilbert (1996). "Evidence that linker sequences and cellulose-binding domains enhance the activity of hemicellulases against complex substrates." Biochem J. **319**(Pt 2): 515-520.
Blake, A. W., L. McCartney, J. E. Flint, D. N. Bolam, A. B. Boraston, H. J. Gilbert and J. P. Knox (2006). "Understanding the biological rationale for the diversity of cellulose-directed carbohydrate-binding modules in prokaryotic enzymes." J Biol Chem **281**(39): 29321-29329.
Blanchette, R. A., E. W. Krueger, J. E. Haight, A. Masood and D. E. Akin (1997). "Cell wall alterations in loblolly pine wood decayed by the white-rot fungus, Ceriporiopsis subvermispora." Journal of Biotechnology **53**(2-3): 203-213.
Bloom, J. D., P. A. Romero, Z. Lu and F. H. Arnold (2007). "Neutral genetic drift can alter promiscuous protein functions, potentially aiding functional evolution." Biol Direct **2**(17).

Blum, D. L., I. A. Kataeva, X.-L. Li and L. G. Ljungdahl (2000). "Feruloyl esterase activity of the Clostridium thermocellum cellulosome can be attributed to previously unknown domains of XynY and XynZ." Journal of Bacteriology **182**(5): 1346-1351.
Boisset, C., C. Fraschini, M. Schulein, B. Henrissat and H. Chanzy (2000). "Imaging the enzymatic digestion of bacterial cellulose ribbons reveals the endo character of the cellobiohydrolase Cel6A from Humicola insolens and its mode of synergy with cellobiohydrolase Cel7A." Appl Environ Microbiol **66**(4): 1444 - 1452.
Bolam, D. N., A. Ciruela, S. McQueen-Mason, P. Simpson, M. P. Williamson, J. E. Rixon, A. Boraston, G. P. Hazlewood and H. J. Gilbert (1998). "Pseudomonas cellulose-binding domains mediate their effects by increasing enzyme substrate proximity." Biochem J **331 (Pt 3)**: 775-781.
Bolam, D. N., H. Xie, G. Pell, D. Hogg, G. Galbraith, B. Henrissat and H. J. Gilbert (2004). "X4 modules represent a new family of carbohydrate-binding modules that display novel properties." J Biol Chem **279**(22): 22953-22963.
Bolvig, P. U., M. Pauly, C. OrWla, H. V. Scheller and K. Schnorr (2003). Sequence analysis and characterisation of a novel pectin acetyl esterase from Bacillus subtilis. Advances in pectins and pectinase research. A. Voragen, H. Schols and R. Visser. Dordrecht, Kluwer Academic Publishers**:** 315-330.
Bomble, Y. J., G. T. Beckham, J. F. Matthews, M. R. Nimlos, M. E. Himmel and M. F. Crowley (2011). "Modeling the self-assembly of the cellulosome enzyme complex." J Biol Chem **286**(7): 5614-5623.
Bonnin, E., K. Clavurier, S. Daniel, S. Kauppinen, J. D. M. Mikkelsen and J.-F. Thibault (2008). "Pectin acetylesterases from Aspergillus are able to deacetylate homogalacturonan as well as rhamnogalacturonan." Carbohydrate Polymers **74**: 411-418.
Boraston, A. B., D. N. Bolam, H. J. Gilbert and G. J. Davies (2004). "Carbohydrate-binding modules: fine-tuning polysaccharide recognition." Biochem. J. **382**(3): 769-781.
Boraston, A. B., B. W. McLean, J. M. Kormos, M. Alam, N. R. Gilkes, C. A. Haynes, P. Tomme, D. G. Kilburn and R. A. J. Warren (1999). Carbohydrate-binding modules: diversity of structure and function. Recent Advances in Carbohydrate Bioengineering. H. J. Gilbert, G. J. Davies, B. Henrissat and B. Svensson. Cambridge, Royal Society of Chemistry**:** 202-211.
Borneman, W. S., L. G. Ljungdahl, R. D. Hartley and D. E. Akin (1991). "Isolation and characterization of p-coumaroyl esterase from the anaerobic fungus Neocallimastix strain MC-2." Appl Environ Microbiol **57**(8): 2337-2344.
Bornscheuer, U. T., G. W. Huisman, R. J. Kazlauskas, S. Lutz, J. C. Moore and K. Robins (2012). "Engineering the third wave of biocatalysis." Nature **485**(7397): 185-194.
Bourbonnais, R. and M. G. Paice (1990). "Oxidation of non-phenolic substrates. An expanded role for laccase in lignin biodegradation." FEBS Lett **267**(1): 99-102.
Brodie, A. F. and F. Lipmann (1955). "Identification of a gluconolactonase." J Biol Chem. **212**(2): 677-685.
Bruchmann, E.-E., H. S. Schach and H. Graf (1987). "Role and properties of lactonase in a cellulase system." Biotech. Appl. Biochem. **9**: 146-159.
Buchert, J., J. Oksanen, J. Pere, M. Siika-Aho, A. Suurnakki and L. Viikari (1998). Applications of Trichoderma reesei enzymes in the pulp and paper industry. Trichoderma and Gliocladium. C. P. Kubicek and G. E. Harman. **1:** 343-364.

Bugg, T. D., M. Ahmad, E. M. Hardiman and R. Rahmanpour (2011). "Pathways for degradation of lignin in bacteria and fungi." Nat Prod Rep **28**(12): 1883-1896.
Burstein, T., M. Shulman, S. Jindou, S. Petkun, F. Frolow, Y. Shoham, E. A. Bayer and R. Lamed (2009). "Physical association of the catalytic and helper modules of a family-9 glycoside hydrolase is essential for activity." FEBS Lett. **583**(5): 879-884
Burton, S. G., D. A. Cowan and J. M. Woodley (2002). "The search for the ideal biocatalyst." Nat Biotechnol **20**(1): 37-45.
Camarero, S., S. Sarkar, F. J. Ruiz-Duenas, M. J. Martinez and A. T. Martinez (1999). "Description of a versatile peroxidase involved in the natural degradation of lignin that has both manganese peroxidase and lignin peroxidase substrate interaction sites." J Biol Chem **274**(15): 10324-10330.
Carrard, G., A. Koivula, H. Söderlund and P. Béguin (2000). "Cellulose-binding domains promote hydrolysis of different sites on crystalline cellulose." Proceedings of the National Academy of Sciences **97**(19): 10342-10347.
Carvalho, A. L., F. M. Dias, T. Nagy, J. A. Prates, M. R. Proctor, N. Smith, E. A. Bayer, G. J. Davies, L. M. Ferreira, M. J. Romao, C. M. Fontes and H. J. Gilbert (2007). "Evidence for a dual binding mode of dockerin modules to cohesins." Proc Natl Acad Sci U S A **104**(9): 3089-3094.
Carvalho, A. L., F. M. Dias, J. A. Prates, T. Nagy, H. J. Gilbert, G. J. Davies, L. M. Ferreira, M. J. Romao and C. M. Fontes (2003). "Cellulosome assembly revealed by the crystal structure of the cohesin-dockerin complex." Proc Natl Acad Sci U S A **100**(24): 13809-13814.
Catcheside, D. E. A., J. P. Rasmussen, P. J. Yeadon, F. J. Bowring, E. B. Cambareri, E. Kato, J. Gabe and W. D. Stuart (2003). "Diversification of exogenous genes in vivo in Neurospora." Applied Microbiology and Biotechnology **62**(5-6): 544-549.
CAZy. (2015). "CAZY database." Retrieved May 20, 2015, from http://www.cazy.org/.
Chauve, M., H. Mathis, D. Huc, D. Casanave, F. Monot and N. L. Ferreira (2010). "Comparative kinetic analysis of two fungal β-glucosidases." Biotechnology for Biofuels **3**(3).
Chen, C.-M., M. Ward, L. Wilson, L. Sumner and S. Shoemaker (1987). "Toward improved cellulases. Targeted modifications of Trichoderma reesei exocellobiohydrolase I/using site-specific mutagenesis." Abstr. Pap. Am. Chem. Soc. **194**.
Chen, K. and F. H. Arnold (1993). "Tuning the activity of an enzyme for unusual environments: sequential random mutagenesis of subtilisin E for catalysis in dimethylformamide." Proc Natl Acad Sci U S A **90**(12): 5618-5622.
Chen, Y., A. J. Stipanovic, W. T. Winter, D. B. Wilson and Y.-J. Kim (2007). "Effect of digestion by pure cellulases on crystallinity and average chain length for bacterial and microcrystalline celluloses." Cellulose **14**(4): 283-293.
Chen, Z. and H. Zhao (2005). "Rapid creation of a novel protein function by in vitro coevolution." J Mol Biol. **348**(5): 1273-1282
Cheng, Y.-S., C.-C. Chen, J.-W. Huang, T.-P. Ko, Z. Huang and R.-T. Guo (2015). "Improving the catalytic performance of a GH11 xylanase by rational protein engineering." Appl Microbiol Biotechnol.
Chir, J. L., C. F. Wan, C. H. Chou and A. T. Wu (2011). "Hydrolysis of cellulose in synergistic mixtures of beta-glucosidase and endo/exocellulase Cel9A from Thermobifida fusca." Biotechnol Lett **33**(4): 777-782.

Cho, N. S., A. Leonowicz, A. Jarosz-Wilkolazka, G. Ginalska, H. Y. Cho and S. J. Shin (2008). "Degradation of a non-phenolic beta-0-4 lignin model dimer by Cerrena unicolor laccase and mediators, acetovanillone and acetosyringone." J Fac Agric Kyushu Univ **53**: 7-12.
Choi, S. K. and L. G. Ljungdahl (1996). "Structural role of calcium for the organization of the cellulosome of Clostridium thermocellum." Biochemistry **35**(15): 4906-4910.
Chundawat, S. P., V. Balan and B. E. Dale (2008). "High-throughput microplate technique for enzymatic hydrolysis of lignocellulosic biomass." Biotechnol Bioeng **99**(6): 1281-1294.
Coffman, A. M., Q. Li and L.-K. Ju (2014). "Effect of natural and pretreated soybean hulls on enzyme production by Trichoderma reesei." Journal of the American Oil Chemists' Society **91**(8): 1331-1338.
Cohen, R., M. R. Suzuki and K. E. Hammel (2005). "Processive endoglucanase active in crystalline cellulose hydrolysis by the brown rot basidiomycete Gloeophyllum trabeum." Appl Environ Microbiol **71**(5): 2412-2417.
Cornet, P., J. Millet, P. Beguin and J.-P. Aubert (1983). "Characterization of two Cel (cellulose degradation) genes of Clostridium Thermocellum coding for endoglucanases." Nat Biotech **1**(7): 589-594.
Coughlan, M. P., K. Hon-Nami, H. Hon-Nami, L. G. Ljungdahl, J. J. Paulin and W. E. Rigsby (1985). "The cellulolytic enzyme complex of Clostridium thermocellum is very large." Biochem Biophys Res Commun **130**(2): 904-909.
Crameri, A., S. A. Raillard, E. Bermudez and W. P. Stemmer (1998). "DNA shuffling of a family of genes from diverse species accelerates directed evolution." Nature **391**(6664): 288-291.
Crawford, D. L., A. L. Pometto and R. L. Crawford (1983). "Lignin degradation by Streptomyces viridosporus: isolation and characterization of a new polymeric lignin degradation intermediate." Appl Environ Microbiol **45**(3): 898-904.
Crestini, C. and D. S. Argyropoulos (1998). "The early oxidative biodegradation steps of residual kraft lignin models with laccase." Bioorganic & Medicinal Chemistry **6**: 2161-2169.
Currie, M. A., J. J. Adams, F. Faucher, E. A. Bayer, Z. Jia and S. P. Smith (2012). "Scaffoldin conformation and dynamics revealed by a ternary complex from the Clostridium thermocellum cellulosome." J Biol Chem **287**(32): 26953-26961.
Currie, M. A., K. Cameron, F. M. Dias, H. L. Spencer, E. A. Bayer, C. M. Fontes, S. P. Smith and Z. Jia (2013). "Small angle X-ray scattering analysis of Clostridium thermocellum cellulosome N-terminal complexes reveals a highly dynamic structure." J Biol Chem **288**(11): 7978-7985.
d'Acunzo, F., C. Galli, P. Gentili and F. Sergi (2006). "Mechanistic and steric issues in the oxidation of phenolic and non-phenolic compounds by laccase or laccase-mediator systems. The case of bifunctional substrates." New Journal of Chemistry **30**(4): 583-591.
Daniel, G., J. Volc and E. Kubatova (1994). "Pyranose oxidase, a major source of H2O2 during wood degradation by Phanerochaete chrysosporium, Trametes versicolor, and Oudemansiella mucida." Appl Environ Microbiol **60**: 2524-2532.
Dassa, B., I. Borovok, R. Lamed, B. Henrissat, P. Coutinho, C. L. Hemme, Y. Huang, J. Zhou and E. A. Bayer (2012). "Genome-wide analysis of Acetivibrio cellulolyticus provides a blueprint of an elaborate cellulosome system." BMC Genomics **13**(1): 210.
Davids, T., M. Schmidt, D. Bottcher and U. T. Bornscheuer (2013). "Strategies for the discovery and engineering of enzymes for biocatalysis." Curr Opin Chem Biol **17**(2): 215-220.

Davidsen, T., E. Beck, A. Ganapathy, R. Montgomery, N. Zafar, Q. Yang, R. Madupu, P. Goetz, K. Galinsky, O. White and G. Sutton (2010). "The comprehensive microbial resource (CMR)." Nucleic Acids Res. **38**: D340-D345
De Groeve, M. R., M. De Baere, L. Hoflack, T. Desmet, E. J. Vandamme and W. Soetaert (2009). "Creating lactose phosphorylase enzymes by directed evolution of cellobiose phosphorylase." Protein Eng Des Sel. **22**(7): 393-399.
de Vries, R. P. and J. Visser (2001). "Aspergillus enzymes involved in degradation of plant cell wall polysaccharides." Microbiol Mol Biol Rev **65**(4): 497-522.
Dekker, R. F. H. (1983). "Bioconversion of hemicellulose: Aspects of hemicellulase production by Trichoderma reesei QM 9414 and enzymic saccharification of hemicellulose." Biotechnology and Bioengineering **25**(4): 1127-1146.
Delgado, L., A. T. Blanco, C. Huitron and G. Aguilar (1992). "Pectin lyase from Aspergillus sp. CH-Y-1043." Applied Microbiology and Biotechnology **39**(4): 515-519.
Demain, A. L., M. Newcomb and J. H. D. Wu (2005). "Cellulase, Clostridia, and ethanol." Microbiology and Molecular Biology Reviews **69**(1): 124-154.
Desjarlais, J. R. and N. D. Clarke (1998). "Computer search algorithms in protein modification and design." Curr Opin Struct Biol. **8**(4): 471-475.
Despotovic, D., L. Vojcic, R. Prodanovic, R. Martinez, K. H. Maurer and U. Schwaneberg (2012). "Fluorescent assay for directed evolution of perhydrolases." J Biomol Screen **17**(6): 796-805.
Dimarogona, M., E. Topakas and P. Christakopoulos (2012). "Cellulose degradation by oxidative enzymes." Comput Struct Biotechnol J. **2**: 1-8.
Din, N., H. G. Damude, N. R. Gilkes, R. C. Miller, Jr., R. A. Warren and D. G. Kilburn (1994). "C1-Cx revisited: intramolecular synergism in a cellulase." Proc Natl Acad Sci U S A **91**(24): 11383-11387.
Ding, S. Y., E. A. Bayer, D. Steiner, Y. Shoham and R. Lamed (1999). "A novel cellulosomal scaffoldin from Acetivibrio cellulolyticus that contains a family 9 glycosyl hydrolase." J Bacteriol. **181**(21): 6720-6729.
Divne, C., J. Stahlberg, T. Reinikainen, L. Ruohonen, G. Pettersson, J. K. Knowles, T. T. Teeri and T. A. Jones (1994). "The three-dimensional crystal structure of the catalytic core of cellobiohydrolase I from Trichoderma reesei." Science **265**(5171): 524-528.
Doucet, N. (2011). "Can enzyme engineering benefit from the modulation of protein motions? Lessons learned from NMR relaxation dispersion experiments." Protein Pept Lett **18**(4): 336-343.
Drevland, R. M., J. W. Cunha, H. Tran, J. Sustarich, P. D. Adams, A. K. Singh, B. A. Simmons and K. L. Sale (2014). Directed evolution of a beta-glucosidase for ionic liquid tolerance. Paper presented at the 36th Symposium on Biotechnology for Fuels and Chemicals, Clearwater Beach, FL.
Drummond, D. A., J. D. Bloom, C. Adami, C. O. Wilke and F. H. Arnold (2005). "Why highly expressed proteins evolve slowly." Proc Natl Acad Sci U S A **102**(40): 14338-14343.
Du, W., Q. Wang, J.-K. Wang and J.-X. Liu (2014). "Enhancing catalytic activity of a xylanase retrieved from a fosmid library of rumen microbiota in hu sheep by directed evolution." Journal of Animal and Veterinary Advances **13**(8): 538-544.

Ďuranová, M., J. Hirsch, K. Kolenova and P. Biely (2009). "Fungal glucuronoyl esterases and substrate uronic acid recognition." Bioscience, biotechnology, and biochemistry **73**(11): 2483-2487.
Dwivedi, U. N., P. Singh, V. P. Pandey and A. Kumar (2011). "Structure–function relationship among bacterial, fungal and plant laccases." Journal of Molecular Catalysis B: Enzymatic **68**(2): 117-128.
Edstrom, R. D. and H. J. Phaff (1963). "Purification and certain properties of pectin trans-eliminase from Aspergihs fonsecaeus* " J Biol Chem. **239**: 2403-2408.
Eneyskaya, E. V., H. Brumer III, L. V. Backinowsky, D. R. Ivanen, A. A. Kulminskaya, K. A. Shabalin and K. N. Neustroev (2003). "Enzymatic synthesis of β-xylanase substrates: transglycosylation reactions of the β-xylosidase from Aspergillus sp." Carbohydrate Research **338**(4): 313-325.
Eneyskaya, E. V., D. R. Ivanen, K. S. Bobrov, L. S. Isaeva-Ivanova, K. A. Shabalin, A. N. Savel'ev, A. M. Golubev and A. A. Kulminskaya (2007). "Biochemical and kinetic analysis of the GH3 family β-xylosidase from Aspergillus awamori X-100." Archives of Biochemistry and Biophysics **457**(2): 225-234.
Eriksen, D. T., J. Lian and H. Zhao (2014). "Protein design for pathway engineering." J. of Struct. Biol. **185**(2): 234–242.
Eriksson, K. E., B. Pettersson and U. Westermark (1974). "Oxidation: an important enzyme reaction in fungal degradation of cellulose." FEBS Lett **49**(2): 282-285.
Escovar-Kousen, J. M., D. Wilson and D. Irwin (2004). "Integration of computer modeling and initial studies of site-directed mutagenesis to improve cellulase activity on Cel9A from Thermobifida fusca." Appl Biochem Biotechnol **113-116**: 287-297.
Evans, C. S., M. V. Dutton, F. Guillén and R. G. Veness (1994). "Enzymes and small molecular mass agents involved with lignocellulose degradation." FEMS Microbiology Reviews **13**(2-3): 235-239.
Fackler, K., C. Gradinger, B. Hinterstoisser, K. Messner and M. Schwanninger (2006). "Lignin degradation by white rot fungi on spruce wood shavings during short-time solid-state fermentations monitored by near infrared spectroscopy." Enzyme and Microbial Technology **39**(7): 1476-1483.
Fägerstam, L. G. and L. G. Pettersson (1980). "The 1,4-β-glucan cellobiohydrolases of Trichoderma reesei QM 9414: A new type of cellulolytic synergism." FEBS Letters **119**(1): 97-100.
Fang, Z., P. Zhou, F. Chang, Q. Yin, W. Fang, J. Yuan, X. Zhang and Y. Xiao (2014). "Structure-based rational design to enhance the solubility and thermostability of a bacterial laccase Lac15." PLoS One **9**(7): e102423.
Fanutti, C., T. Ponyi, G. W. Black, G. P. Hazlewood and H. J. Gilbert (1995). "The conserved noncatalytic 40-residue sequence in cellulases and hemicellulases from anaerobic fungi functions as a protein docking domain." Journal of Biological Chemistry **270**(49): 29314-29322.
Fauth, U., M. P. Romaniec, T. Kobayashi and A. L. Demain (1991). "Purification and characterization of endoglucanase Ss from Clostridium thermocellum." Biochem. J. **279**(1): 67-70.
Fernandez-Alvaro, E., R. Snajdrova, H. Jochens, T. Davids, D. Bottcher and U. T. Bornscheuer (2011). "A combination of in vivo selection and cell sorting for the identification of enantioselective biocatalysts." Angew Chem Int Ed Engl **50**(37): 8584-8587.

Ferre, H., A. Broberg, J. O. Duus and K. K. Thomsen (2000). "A novel type of arabinoxylan arabinofuranohydrolase isolated from germinated barley analysis of substrate preference and specificity by nano-probe NMR." Eur J Biochem **267**(22): 6633-6641.
Ferrés, I., V. Amarelle, F. Noya and E. Fabiano (2015). "Identification of Antarctic culturable bacteria able to produce diverse enzymes of potential biotechnological interest " Advances in Polar Science **26**(1): 71-79.
Fierobe, H.-P., A. Mechaly, C. Tardif, A. Belaich, R. Lamed, Y. Shoham, J.-P. Belaich and E. A. Bayer (2001). "Design and production of active cellulosome chimeras. Selective incorporation of dockerin-containing enzymes into defined functional complexes." Journal of Biological Chemistry **276**(24): 21257-21261.
Fierobe, H.-P., F. Mingardon, A. Mechaly, A. Bélaïch, M. T. Rincon, S. Pagès, R. Lamed, C. Tardif, J.-P. Bélaïch and E. A. Bayer (2005). "Action of designer cellulosomes on homogeneous versus complex substrates - Controlled incorporation of three distinct enzymes into a defined trifunctional scaffoldin." J Biol Chem **280**(16): 16325-16334.
Fierobe, H.-P., F. Mingardon, A. Mechaly, A. Bélaïch, M. T. Rincon, S. Pagès, R. Lamed, C. Tardif, J.-P. Bélaïch and E. A. Bayer (2005). "Action of designer cellulosomes on homogeneous versus complex substrates: controlled incorporation of three distinct enzymes into a defined trifunctional scaffoldin." Journal of Biological Chemistry **280**(16): 16325-16334.
Fierobe, H. P., E. A. Bayer, C. Tardif, M. Czjzek, A. Mechaly, A. Belaich, R. Lamed, Y. Shoham and J. P. Belaich (2002). "Degradation of cellulose substrates by cellulosome chimeras. Substrate targeting versus proximity of enzyme components." J Biol Chem **277**(51): 49621-49630.
Fierobe, H. P., S. Pagès, A. Bélaïch, S. Champ, D. Lexa and J. P. Bélaïch (1999). "Cellulosome from Clostridium cellulolyticum: molecular study of the Dockerin/Cohesin interaction." Biochemistry **38**(39): 12822-12832.
Fillingham, I. J., P. A. Kroon, G. Williamson, H. J. Gilbert and G. P. Hazlewood (1999). "A modular cinnamoyl ester hydrolase from the anaerobic fungus Piromyces equi acts synergistically with xylanase and is part of a multiprotein cellulose-binding cellulase-hemicellulase complex." Biochem. J. **343**(1): 215-224.
Flournoy, D. S., J. A. Paul, T. K. Kirk and T. L. Highley (1993). "Changes in the size and volume of pores in sweetgum wood during simultaneous rot by Phanerochaete chrysosporium burds." Holzforschung **47**: 297-301.
Fonseca-Maldonado, R., D. S. Vieira, J. S. Alponti, E. Bonneil, P. Thibault and R. J. Ward (2013). "Engineering the pattern of protein glycosylation modulates the thermostability of a GH11 xylanase." Journal of Biological Chemistry **288**(35): 25522-25534.
Fontes, C. M., G. P. Hazlewood, E. Morag, J. Hall, B. H. Hirst and H. J. Gilbert (1995). "Evidence for a general role for non-catalytic thermostabilizing domains in xylanases from thermophilic bacteria." Biochem J **307 (Pt 1)**: 151-158.
Fontes, C. M. G. A. and H. J. Gilbert (2010). "Cellulosomes: highly efficient nanomachines designed to deconstruct plant cell wall complex carbohydrates." Annual Review of Biochemistry **79**(1): 655-681.
Foreman, P., D. Brown, L. Dankmeyer, R. Dean, S. Diener, N. Dunn-Coleman, F. Goedegebuur, T. Houfek, G. England and A. Kelley (2003). "Transcriptional regulation of biomass-degrading enzymes in the filamentous fungus Trichoderma reesei." J Biol Chem **278**: 31988 - 31997.

Foreman, P., D. Brown, L. Dankmeyer, R. Dean, S. Diener, N. Dunn-Coleman, F. Goedegebuur, T. Houfek, G. England, A. Kelley, H. Meerman, T. Mitchell, C. Mitchinson, H. Olivares, P. Teunissen, J. Yao and M. Ward (2003). "Transcriptional regulation of biomass-degrading enzymes in the filamentous fungus Trichoderma reesei." J Biol Chem **278**: 31988 - 31997.
Foreman, P. K., D. Brown, L. Dankmeyer, R. Dean, S. Diener, N. S. Dunn-Coleman, F. Goedegebuur, T. D. Houfek, G. J. England, A. S. Kelley, H. J. Meerman, T. Mitchell, C. Mitchinson, H. A. Olivares, P. J. M. Teunissen, J. Yao and M. Ward (2003). "Transcriptional regulation of biomass-degrading enzymes in the filamentous fungus Trichoderma reesei." Journal of Biological Chemistry **278**(34): 31988-31997.
Forsberg, Z., G. Vaaje-Kolstad, B. Westereng, A. C. Bunaes, Y. Stenstrom, A. MacKenzie, M. Sorlie, S. J. Horn and V. G. Eijsink (2011). "Cleavage of cellulose by a CBM33 protein." Protein Sci **20**(9): 1479-1483.
Fowler, T. and R. D. Brown (1992). "The bgl1 gene encoding extracellular β-glucosidase from Trichoderma reesei is required for rapid induction of the cellulase complex." Molecular Microbiology **6**(21): 3225-3235.
Fox, R. J., S. C. Davis, E. C. Mundorff, L. M. Newman, V. Gavrilovic, S. K. Ma, L. M. Chung, C. Ching, S. Tam, S. Muley, J. Grate, J. Gruber, J. C. Whitman, R. A. Sheldon and G. W. Huisman (2007). "Improving catalytic function by ProSAR-driven enzyme evolution." Nat Biotechnol. **25**(3): 338-344.
Franke, W. and M. Deffner (1939). "Zur Kenntnis der sog. Glucose-oxydase. II." Liebigs Ann. **541**(1): 117-150.
Franke, W. and F. Lorenz (1937). "Zur Kenntnis der sog. Glucose-oxydase. I." Liebigs Ann. **532**(1): 1-28.
Freer, S. N. (1993). "Kinetic Characterization of a β-Glucosidase from a Yeast, Candida wickerhamii." The journal of biological chemestry **268**(13): 9337-9342.
Fries, M., J. Ihrig, K. Brocklehurst, V. E. Shevchik and R. W. Pickersgill (2007). "Molecular basis of the activity of the phytopathogen pectin methylesterase." EMBO J. **26**(17): 3879-3887.
Fujii, T., Yu, G., A. Matsushika, A. Kurita, S. Yano, K. Murakami and S. Sawayama (2011). "Ethanol production from xylo-oligosaccharides by xylose-fermenting Saccharomyces cerevisiae expressing β-xylosidase." Bioscience, biotechnology, and biochemistry **75**(6): 1140-1146.
Fujino, T., P. Béguin and J. P. Aubert (1992). "Cloning of a Clostridium thermocellum DNA fragment encoding polypeptides that bind the catalytic components of the cellulosome." FEMS Microbiol Lett. **73**(1-2): 165-170.
Furukawa, T., F. O. Bello and L. Horsfall (2014). "Microbial enzyme systems for lignin degradation and their transcriptional regulation." Frontiers in Biology **9**(6): 448-471.
Fushinobu, S., M. Hidaka, Y. Honda, T. Wakagi, H. Shoun and M. Kitaoka (2005). "Structural basis for the specificity of the reducing end xylose-releasing exo-oligoxylanase from Bacillus halodurans C-125." J Biol Chem **280**(17): 17180-17186.
Galante, Y. M., A. De Conti and R. Monteverdi (1998). Application of Trichoderma enzymes in the textile industry. Trichoderma and Gliocladium. G. E. Harman and C. P. Kubicek. London, Taylor and Francis. **1:** 311-326.
Galkin, S., T. Vares, M. Kalsi and A. Hatakka (1998). "Production of organic acids by different white-rot fungi
as detected using capillary zone electrophoresis." Biotechnol Technol **12**: 267-271.

Ganju, R. K., P. J. Vithayathil and S. K. Murthy (1989). "Purification and characterization of two xylanases from Chaetomium thermophile var. coprophile." Canadian journal of microbiology **35**(9): 836-842.
Garcia-Alvarez, B., R. Melero, F. M. Dias, J. A. Prates, C. M. Fontes, S. P. Smith, M. J. Romao, A. L. Carvalho and O. Llorca (2011). "Molecular architecture and structural transitions of a Clostridium thermocellum mini-cellulosome." J Mol Biol **407**(4): 571-580.
Garcia-Martinez, D. V., A. Shinmyo, A. Madia and A. L. Demain (1980). "Studies on cellulase production by Clostridium thermocellum." European journal of applied microbiology and biotechnology **9**(3): 189-197.
Garcia-Ruiz, E., D. Gonzalez-Perez, F. J. Ruiz-Dueñas, A. T. Martínez and M. Alcalde (2012). "Directed evolution of a temperature-, peroxide- and alkaline pH-tolerant versatile peroxidase." Biochemical Journal **441**(1): 487-498.
Gasparic, A., J. Martin, A. S. Daniel and H. J. Flint (1995). "A xylan hydrolase gene cluster in Prevotella ruminicola B(1)4: sequence relationships, synergistic interactions, and oxygen sensitivity of a novel enzyme with exoxylanase and beta-(1,4)-xylosidase activities." Appl Environ Microbiol **61**(8): 2958-2964.
Gerlt, J. A. and P. C. Babbitt (2009). "Enzyme (re)design: lessons from natural evolution and computation." Curr Opin Chem Biol **13**(1): 10-18.
Gerngross, U. T., M. P. Romaniec, T. Kobayashi, N. S. Huskisson and A. L. Demain (1993). "Sequencing of a Clostridium thermocellum gene (cipA) encoding the cellulosomal SL-protein reveals an unusual degree of internal homology." Mol Microbiol. **8**(2): 325-334.
Gerwig, G. J., P. de Waard, J. P. Kamerling, J. F. G. Vliegenthart, E. Morgenstern, R. Lamed and E. A. Bayer (1989). "Novel O-linked carbohydrate chains in the cellulase complex (cellulosome) of Clostridium thermocellum. 3-O-Methyl-N-acetylglucosamine as a constituent of a glycoprotein." J. Biol. Chem. **264**(2): 1027-1035.
Gerwig, G. J., J. P. Kamerling, J. F. G. Viliegentthart, E. Morag, R. Lamed and E. A. Bayer (1992). "Novel oligosaccharide constituents of the cellulase complex of Bacteroides cellulosolvens." Eur. J. Biochem. **205**(2): 799-808.
Gerwig, G. J., J. P. Kamerling, J. F. Vliegenthart, E. Morag, R. Lamed and E. A. Bayer (1991). "Primary structure of O-linked carbohydrate chains in the cellulosome of different Clostridium thermocellum strains." Eur. J. Biochem. **196**(1): 115-122.
Gerwig, G. J., J. P. Kamerling, J. F. Vliegenthart, E. Morag, R. Lamed and E. A. Bayer (1993). "The nature of the carbohydrate-peptide linkage region in glycoproteins from the cellulosomes of Clostridium thermocellum and Bacteroides cellulosolvens." Journal of Biological Chemistry **268**(36): 26956-26960.
Gilad, R., L. Rabinovich, S. Yaron, E. A. Bayer, R. Lamed, H. J. Gilbert and Y. Shoham (2003). "CelI, a noncellulosomal family 9 enzyme from Clostridium thermocellum, is a processive endoglucanase that degrades crystalline cellulose." J Bacteriol **185**(2): 391-398.
Gilbert, H. J. (2007). "Cellulosomes: microbial nanomachines that display plasticity in quaternary structure." Mol Microbiol **63**(6): 1568-1576.
Gilbert, H. J., J. P. Knox and A. B. Boraston (2013). "Advances in understanding the molecular basis of plant cell wall polysaccharide recognition by carbohydrate-binding modules." Curr Opin Struct Biol. **23**(5): 669-677.

Gilkes, N. R., M. L. Langsford, D. G. Kilburn, J. Miller, Robert C. and R. A. J. Warren (1984). "Mode of action and substrate specificities of cellulases from cloned bacterial genes." J Biol Chem. **259**(16): 10455-10459.
Gilkes, N. R., R. A. Warren, R. C. J. Miller and D. G. Kilburn (1988). "Precise excision of the cellulose binding domains from two Cellulomonas fimi cellulases by a homologous protease and the effect on catalysis." J Biol Chem. **263**(21): 10401-10407.
Gilkes, N. R., R. A. Warren, R. C. J. Miller and D. G. Kilburn (1988). "Precise excision of the cellulose binding domains from two Cellulomonas fimi cellulases by a homologous protease and the effect on catalysis." J Biol Chem. **263**(21): 10401-10407.
Glenn, J. K., L. Akileswaran and M. H. Gold (1986). "Mn(II) oxidation is the principal function of the extracellular Mn-peroxidase from Phanerochaete chrysosporium." Archives of Biochemistry and Biophysics **251**(2): 688-696.
Glenn, J. K. and M. H. Gold (1985). "Purification and characterization of an extracellular Mn(II)-dependent peroxidase from the lignin-degrading basidiomycete, Phanerochaete chrysosporium." Archives of Biochemistry and Biophysics **242**(2): 329-341.
Glenn, J. K. and M. H. Gold (1985). "Purification and characterization of an extracellular Mn(II)-dependent peroxidase from the lignin-degrading basidiomycete, Phanerochaete chrysosporium." Arch Biochem Biophys **242**(2): 329-341.
Glieder, A., E. T. Farinas and F. H. Arnold (2002). "Laboratory evolution of a soluble, self-sufficient, highly active alkane hydroxylase." Nat Biotechnol **20**(11): 1135-1139.
Glumoff, T., P. J. Harvey, S. Molinari, M. Goble, G. Frank, J. M. Palmer, J. D. G. Smit and M. S. A. Leisola (1990). "Lignin peroxidase from Phanerochaete chrysosporium." Eur. J. Biochem. **187**(3): 515-520
Goldberg, R. N. (1975). "Thermodynamics of hexokinase-catalyzed reactions." Biophysical Chemistry **3**(3): 192-205.
Gomez-Toribio, V., A. T. Martinez, M. J. Martinez and F. Guillen (2001). "Oxidation of hydroquinones by the versatile ligninolytic peroxidase from Pleurotus eryngii. H2O2 generation and the influence of Mn2+." Eur J Biochem **268**(17): 4787-4793.
González-Blasco, G., J. Sanz-Aparicio, B. González, J. A. Hermoso and J. Polaina (2000). "Directed evolution of β-Glucosidase a from Paenibacillus polymyxa to thermal resistance." Journal of Biological Chemistry **275**(18): 13708-13712.
Gosh, B. K. and A. Gosh (1992). Degradation of cellulose by fungal cellulase. Microbial Degradation of Natural Products. G. Winkelmann. New York, VCH Publishers Inc.**:** 84-126.
Gräbnitz, F., R. K. P., M. Seiss and W. L. Staudenbauer (1991). "Structure of the β-glucosidase gene bglA of Clostridium thermocellum." European Journal of Biochemistry **200**(2): 301-309.
Gräbnitz, F., K. P. Rücknagel, M. Seiß and W. L. Staudenhauer (1989). "Nucleotide sequence of the Clostridium thermocellum bglB gene encoding thermostable β-glucosidase B: Homology to fungal β-glucosidases." Molecular and General Genetics MGG **217**(1): 70-76.
Grépinet, O. and P. Béguin (1986). "Sequence of the cellulase gene of Clostridium thermoceltum coding for endoglucanase B." Nucl. Acids Res. **14**(4): 1791-1799.
Grépinet, O., M. C. Chebrou and P. Béguin (1988). "Purification of Clostridium thermocellum xylanase Z expressed in Escherichia coli and identification of the corresponding product in the culture medium of C. thermocellum." Journal of Bacteriology **170**(10): 4576-4581.

Griffiths, J. S., M. Cheriyan, J. B. Corbell, L. Pocivavsek, C. A. Fierke and E. J. Toone (2004). "A bacterial selection for the directed evolution of pyruvate aldolases." Bioorganic & Medicinal Chemistry **12**(15): 4067-4074.
Grishutin, S., A. Gusakov, A. Markov, B. Ustinov, M. Semenova and A. Sinitsyn (2004). "Specific xyloglucanases as a new class of polysaccharide-degrading enzymes." Biochim Biophys Acta **1674**: 268 - 281.
Grishutin, S. G., A. V. Gusakov, A. V. Markov, B. B. Ustinov, M. V. Semenova and A. P. Sinitsyn (2004). "Specific xyloglucanases as a new class of polysaccharide-degrading enzymes." Biochimica et Biophysica Acta (BBA) - General Subjects **1674**(3): 268-281.
Gübitz, G. M., M. Hayn, M. Sommerauer and W. Steiner (1996). "Mannan-degrading enzymes from Sclerotium rolfsii: Characterisation and synergism of two endo β-mannanases and a β-mannosidase." Bioresource Technology **58**(2): 127-135.
Guenther, C. M., B. E. Kuypers, M. T. Lam, T. M. Robinson, J. Zhao and J. Suh (2014). "Synthetic virology: engineering viruses for gene delivery." Wiley Interdiscip Rev Nanomed Nanobiotechnol. **6**(6): 548-558.
Guillén, F., A. T. Martinez and M. J. Martínez (1990). "Production of hydrogen peroxide by aryl-alcohol oxidase from the ligninolytic fungus Pleurotus eryngii." Appl. Microbioi. Biotechnol. Bioeng. **32**(4): 465-469
Guillen, F., M. J. Martinez, C. Munoz and A. T. Martinez (1997). "Quinone redox cycling in the ligninolytic fungus Pleurotus eryngii leading to extracellular production of superoxide anion radical." Arch Biochem Biophys **339**(1): 190-199.
Guo, H. H., J. Choe and L. A. Loeb (2004). "Protein tolerance to random amino acid change." Proc Natl Acad Sci U S A **101**(25): 9205-9210.
Gupta, N. and E. T. Farinas (2010). "Directed evolution of CotA laccase for increased substrate specificity using Bacillus subtilis spores." Protein Eng Des Sel. **23**(8): 679-682.
Gupta, R. D. and D. S. Tawfik (2008). "Directed enzyme evolution via small and effective neutral drift libraries." Nat Methods **5**(11): 939-942.
Gusakov, A. V. and A. P. Sinitsyn (1992). "A theoretical analysis of cellulase product inhibition: effect of cellulase binding constant, enzyme/substrate ratio, and beta-glucosidase activity on the inhibition pattern." Biotechnol Bioeng. **40**(6): 663-671.
Gutierrez, A., L. Caramelo, A. Prieto, M. J. Martinez and A. T. Martinez (1994). "Anisaldehyde production and aryl-alcohol oxidase and dehydrogenase activities in ligninolytic fungi of the genus Pleurotus." Appl Environ Microbiol **60**(6): 1783-1788.
Haitjema, C., K. Solomon and M. A. O'Malley (2013). Biochemical insight into fungal cellulosome architecture and regulation. AIChE Annual Meeting. San Francisco, CA.
Hakkinen, M., M. Arvas, M. Oja, N. Aro, M. Penttila, M. Saloheimo and T. Pakula (2012). "Re-annotation of the CAZy genes of Trichoderma reesei and transcription in the presence of lignocellulosic substrates." Microbial Cell Factories **11**(134).
Hall, J., G. P. Hazlewood, P. J. Barker and H. J. Gilbert (1988). "Conserved reiterated domains in Clostridium thermocellum endoglucanases are not essential for catalytic activity." Gene **69**(1): 29-38.
Halstead, J. R., P. E. Vercoe, H. J. Gilbert, K. Davidson and G. P. Hazlewood (1999). "A family 26 mannanase produced by Clostridium thermocellum as a component of the cellulosome

contains a domain which is conserved in mannanases from anaerobic fungi." Microbiology **145**(11): 3101-3108.
Hammel, K. E., A. N. Kapich, K. A. Jensen Jr and Z. C. Ryan (2002). "Reactive oxygen species as agents of wood decay by fungi." Enzyme and Microbial Technology **30**(4): 445-453.
Hammel, K. E., M. Tien, B. Kalyanaraman and T. K. Kirk (1985). "Mechanism of oxidative C alpha-C beta cleavage of a lignin model dimer by Phanerochaete chrysosporium ligninase. Stoichiometry and involvement of free radicals." J Biol Chem **260**(14): 8348-8353.
Hammel, M., H. P. Fierobe, M. Czjzek, S. Finet and V. Receveur-Brechot (2004). "Structural insights into the mechanism of formation of cellulosomes probed by small angle X-ray scattering." J Biol Chem **279**(53): 55985-55994.
Hammel, M., H. P. Fierobe, M. Czjzek, V. Kurkal, J. C. Smith, E. A. Bayer, S. Finet and V. Receveur-Brechot (2005). "Structural basis of cellulosome efficiency explored by small angle X-ray scattering." J Biol Chem **280**(46): 38562-38568.
Handelsman, J., M. R. Rondon, S. F. Brady, J. Clardy and R. M. Goodman (1998). "Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products." Chem Biol. **5**(10): R245-249.
Hardiman, E., M. Gibbs, R. Reeves and P. Bergquist (2010). "Directed evolution of a thermophilic beta-glucosidase for cellulosic bioethanol production." Appl Biochem Biotechnol **161**(1-8): 301-312.
Harkin, J. M. and J. R. Obst (1974). "Demethylation of 2,4,6-trimethoxyphenol by phenol oxidases: a model for chromophore formation in wood and pulp." Tappi J **57**(7): 118-121
Harris, P. V., D. Welner, K. C. McFarland, E. Re, J.-C. Navarro Poulsen, K. Brown, R. Salbo, H. Ding, E. Vlasenko, S. Merino, F. Xu, J. Cherry, S. Larsen and L. Lo Leggio (2010). "Stimulation of Lignocellulosic Biomass Hydrolysis by Proteins of Glycoside Hydrolase Family 61: Structure and Function of a Large, Enigmatic Family." Biochemistry **49**(15): 3305-3316.
Harvey, P. J., H. E. Schoemaker, R. M. Bowen and J. M. Palmer (1985). "Single-electron transfer processes and the reaction mechanism of enzymic degradation of lignin." FEBS Letters **183**(1): 13-16.
Hayashi, H., K. I. Takagi, M. Fukumura, T. Kimura, S. Karita, K. Sakka and K. Ohmiya (1997). "Sequence of xynC and properties of XynC, a major component of the Clostridium thermocellum cellulosome." Journal of Bacteriology **179**(13): 4246-4253.
Hayashi, H., M. Takehara, T. Hattori, T. Kimura, S. Karita, K. Sakka and K. Ohmiya (1999). "Nucleotide sequences of two contiguous and highly homologous xylanase genes xynA and xynB and characterization of XynA from Clostridium thermocellum." Appl Microbiol Biotechnol. **51**(3): 348-357.
Hazlewood, G. P., K. Davidson, J. H. Clarke, A. J. Durrant, J. Hall and H. J. Gilbert (1990). "Endoglucanase E, produced at high level in Escherichia coli as a lacZ' fusion protein, is part of the Clostridium thermocellum cellulosome." Enzyme and Microbial Technology **12**(9): 656-662.
Hebraud, M. and M. Fevre (1990). "Purification and characterization of an extracellular beta-xylosidase from the rumen anaerobic fungus Neocallimastix frontalis." FEMS Microbiol Lett. **60**(1-2): 11-16.
Heinzelman, P., R. Komor, A. Kanaan, P. Romero, X. Yu, S. Mohler, C. Snow and F. Arnold (2010). "Efficient screening of fungal cellobiohydrolase class I enzymes for thermostabilizing sequence blocks by SCHEMA structure-guided recombination." Protein Eng Des Sel **23**(11): 871-880.

Heinzelman, P., C. D. Snow, M. A. Smith, X. Yu, A. Kannan, K. Boulware, A. Villalobos, S. Govindarajan, J. Minshull and F. H. Arnold (2009b). "SCHEMA recombination of a fungal cellulase uncovers a single mutation that contributes markedly to stability." J Biol Chem **284**(39): 26229-26233.
Heinzelman, P., C. D. Snow, I. Wu, C. Nguyen, A. Villalobos, S. Govindarajan, J. Minshull and F. H. Arnold (2009a). "A family of thermostable fungal cellulases created by structure-guided recombination." Proc Natl Acad Sci U S A **106**(14): 5610-5615.
Henriksson, G., G. Johansson and G. Pettersson (2000). "A critical review of cellobiose dehydrogenases." J Biotechnol **78**(2): 93-113.
Henriksson, G., A. Nutt, H. Henriksson, B. Pettersson, J. Stahlberg, G. Johansson and G. Pettersson (1999). "Endoglucanase 28 (Cel12A), a new Phanerochaete chrysosporium cellulase." Eur J Biochem **259**(1-2): 88-95.
Henriksson, G., G. Pettersson, G. Johansson, A. Ruiz and E. Uzcategui (1991). "Cellobiose oxidase from Phanerochaete chrysosporium can be cleaved by papain into two domains." Eur J Biochem. **196**(1): 101-106.
Henrissat, B., H. Driguez, C. Viet and M. Schulein (1985). "Synergism of cellulases from Trichoderma reesei in the degradation of cellulose." Nat Biotech **3**(8): 722-726.
Hernandez-Ortega, A., P. Ferreira and A. T. Martinez (2012). "Fungal aryl-alcohol oxidase: a peroxide-producing flavoenzyme involved in lignin degradation." Appl Microbiol Biotechnol **93**(4): 1395-1410.
Hernandez, K. and R. Fernandez-Lafuente (2011). "Control of protein immobilization: coupling immobilization and site-directed mutagenesis to improve biocatalyst or biosensor performance." Enzyme Microb Technol **48**(2): 107-122.
Herpoel-Gimbert, I., A. Margeot, A. Dolla, G. Jan, D. Molle, S. Lignon, H. Mathis, J.-C. Sigoillot, F. Monot and M. Asther (2008). "Comparative secretome analyses of two Trichoderma reesei RUT-C30 and CL847 hypersecretory strains." Biotechnology for Biofuels **1**(1): 18.
Herrmann, M. C., M. Vrsanska, M. Jurickova, J. Hirsch, P. Biely and C. P. Kubicek (1997). "The beta-D-xylosidase of Trichoderma reesei is a multifunctional beta-D-xylan xylohydrolase." Biochem J. **321**(Pt 2): 375-381.
Hervé, C., A. Rogowski, A. W. Blake, S. E. Marcus, H. J. Gilbert and J. P. Knox (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**(34): 15293-15298.
Hjortkjaer, R. K., V. Bille-Hansen, K. P. Hazelden, M. McConville, D. B. McGregor, J. A. Cuthbert, R. J. Greenough, E. Chapman, J. R. Gardner and R. Ashby (1986). "Safety evaluation of celluclast®, an acid cellulase derived from Trichoderma reesei." Food and Chemical Toxicology **24**(1): 55-63.
Hokanson, C. A., G. Cappuccilli, T. Odineca, M. Bozic, C. A. Behnke, M. Mendez, W. J. Coleman and R. Crea (2011). "Engineering highly thermostable xylanase variants using an enhanced combinatorial library method." Protein Eng Des Sel **24**(8): 597-605.
Holtzapple, M., M. Cognata, Y. Shu and C. Hendrickson (1990). "Inhibition of Trichoderma reesei cellulase by sugars and solvents." Biotechnol. Bioeng. **36**(3): 275-287.
Holtzapple, M. T., H. S. Caram and A. E. Humphrey (1984). "Determining the inhibition constants in the HCH-1 model of cellulose hydrolysis." Biotechnol Bioeng. **26**(7): 753-757.

Honda, Y. and M. Kitaoka (2004). "A family 8 glycoside hydrolase from Bacillus halodurans C-125 (BH2105) is a reducing end xylose-releasing exo-oligoxylanase." J Biol Chem **279**(53): 55097-550103.
Hong, M. R., C. S. Park and D. K. Oh (2009). "Characterization of a thermostable endo-1,5-alpha-L-arabinanase from Caldicellulorsiruptor saccharolyticus." Biotechnol Lett. **31**(9): 1439-1443.
Hsieh, C. W., D. Cannella, H. Jørgensen, C. Felby and L. G. Thygesen (2014). "Cellulase inhibition by high concentrations of monosaccharides." J Agric Food Chem. **62**(17): 3800-3005.
Hu, J., R. P. Chandra, V. Arantes, K. Gourlay, J. S. v. Dyk and J. N. Saddler (2015). "The addition of accessory enzymes enhances the hydrolytic performance of cellulase enzymes at high solid loadings." Bioresource Technology **186**: 149–153.
Huang, J.-W., C.-C. Chen, C.-H. Huang, T.-Y. Huang, T.-H. Wu, Y.-S. Cheng, T.-P. Ko, C.-Y. Lin, J.-R. Liu and R.-T. Guo (2014). "Improving the specific activity of β-mannanase from Aspergillus niger BK01 by structure-based rational design." Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics **1844**(3): 663-669.
Hudlicky, T. and J. W. Reed (2009). "Applications of biotransformations and biocatalysis to complexity generation in organic synthesis." Chem Soc Rev **38**(11): 3117-3132.
Ibrahim, V., L. Mendozaa, G. Mamo and R. Hatti-Kaul (2011). "Blue laccase from Galerina sp.: Properties and potential for Kraft lignin demethylation." Process Biochemistry **46**(1): 379-384.
Igarashi, K., A. Koivula, M. Wada, S. Kimura, M. Penttilä and M. Samejima (2009). "High Speed Atomic Force Microscopy Visualizes Processive Movement of Trichoderma reesei Cellobiohydrolase I on Crystalline Cellulose." Journal of Biological Chemistry **284**(52): 36186-36190.
Igarashi, K., M. Samejima and K. E. L. Eriksson (1998). "Cellobiose dehydrogenase enhances Phanerochaete chrysosporium cellobiohydrolase I activity by relieving product inhibition." Eur J Biochem **253**(1): 101-106.
Igarashi, K., T. Uchihashi, A. Koivula, M. Wada, S. Kimura, T. Okamoto, M. Penttilä, T. Ando and M. Samejima (2011). "Traffic jams reduce hydrolytic efficiency of cellulase on cellulose surface." Science **333**(6047): 1279-1282.
Igual, J. M., E. Velázquez, P. F. Mateos, C. Rodríguez-Barrueco, E. Cervantes and E. Martínez-Molina (2001). "Cellulase isoenzyme profiles in Frankia strains belonging to different cross-inoculation groups." Plant and Soil **229**(1): 35-39.
Irwin, D., D.-H. Shin, S. Zhang, B. K. Barr, J. Sakon, P. A. Karplus and D. B. Wilson (1998). "Roles of the catalytic domain and two cellulose binding domains of Thermomonospora fusca E4 in cellulose hydrolysis." Journal of Bacteriology **180**(7): 1709-1714.
Ishihara, T. and M. Miyaxaki (1974). "Demethylation of lignin and lignin models by fungal laccase " Mokurai Gakkaishi **20**(1): 39.
Ishihara, T. and M. Miyaxaki (1976). "Oxidation of syringic acid beta oxidation of syringic acid by fungal laccase y fungal laccase." Mokuzai Gakkoishi **22**: 371.
Jamal-Talabani, S., A. B. Boraston, J. P. Turkenburg, N. Tarbouriech, V. M. A. Ducros and G. J. Davies (2004). "Ab Initio structure determination and functional characterization of CBM36: a new family of calcium-dependent carbohydrate binding modules." Structure **12**(7): 1177-1187.
Janssen, F. W., R. M. Kerwin and H. W. Ruelius (1965). "Alcohol oxidase, a novel enzyme from a basidiomycete." Biochem Biophys Res Commun. **20**(5): 630-634.

Janssen, F. W. and H. W. Ruelius (1968a). "Carbohydrate oxidase, a novel enzyme from polyporus obtusus: II. Specificity and characterization of reaction products." Biochim Biophys Acta **167**(3): 501-510.
Janssen, F. W. and H. W. Ruelius (1968b). "Alcohol oxidase, a flavoprotein from several Basidiomycetes species. Crystallization by fractional precipitation with polyethylene glycol." Biochim. Biophys. Acta **151**: 303-342.
Jensen, K. A., W. Bao, S. Kawai, E. Srebotnik and K. E. Hammel (1996). "Manganese-dependent cleavage of nonphenolic lignin structures by Ceriporiopsis subvermispora in the absence of lignin peroxidase." Applied and Environmental Microbiology **62**(10): 3679-3686.
Ji, L., J. Yang, H. Fan, Y. Yang, B. Li, X. Yu, N. Zhu and H. Yuan (2014). "Synergy of crude enzyme cocktail from cold-adapted Cladosporium cladosporioides Ch2-2 with commercial xylanase achieving high sugars yield at low cost." Biotechnology for Biofuels **7**(130).
Jimenéz-Zurdo, J. I., P. F. Mateos, F. B. Dazzo and E. Martínez-Molina (1996). "Cell-bound cellulase and polygaracturonase production by Rhizobium and Bradyrhizobium species." Soil Biology and Biochemistry **28**(7): 917-921.
Jindou, S., A. Soda, S. Karita, T. Kajino, P. Béguin, J. H. D. Wu, M. Inagaki, T. Kimura, K. Sakka and K. Ohmiya (2004). "Cohesin-dockerin interactions within and between Clostridium josui and Clostridium thermocellum: binding seectivity between cognate dockerin and cohesin domains and species specificity." Journal of Biological Chemistry **279**(11): 9867-9874.
Jochens, H. and U. T. Bornscheuer (2010). "Natural diversity to guide focused directed evolution." Chembiochem **11**(13): 1861-1866.
Johansson, T., K. G. Welinder and P. O. Nyman (1993). "Isozymes of lignin peroxidase and manganese(II) peroxidase from the white-rot Basidiomycete Trametes versicolor: II. Partial sequences, peptide maps, and amino acid and carbohydrate compositions." Archives of Biochemistry and Biophysics **300**(1): 57-62.
Johnson, D. L., J. L. Thompson, S. M. Brinkmann, K. A. Schuller and L. L. Martin (2003). "Electrochemical characterization of purified Rhus vernicifera Laccase: voltammetric evidence for a sequential four-electron transfer." Biochemistry **42**: 10229-10237.
Johnson, E. A., M. Sakajoh, G. Halliwell, A. Madia and A. L. Demain (1982). "Saccharification of complex cellulosic substrates by the cellulase system from Clostridium thermocellum." Applied and Environmental Microbiology **43**(5): 1125-1132.
Johnsson, K., R. K. Allemann, H. Widmer and S. A. Benner (1993). "Synthesis, structure and activity of artificial, rationally designed catalytic polypeptides." Nature **365**(6446): 530-532.
Joliff, G., P. Béguin and J.-P. Aubert (1986a). "Nucleotide sequence of the cellulase gene celD encoding endoglucanase D of Clostridium thermocellum." Nucleic Acids Research **14**(21): 8605-8612.
Joliff, G., P. Beguin, M. Juy, J. Millet, A. Ryter, R. Poljak and J.-P. Aubert (1986b). "Isolation, crystallization and properties of a new cellulase of Clostridium thermocellum overproduced in Escherichia coli." Nat Biotech **4**(10): 896-900.
Joo, J. C., S. P. Pack, Y. H. Kim and Y. J. Yoo (2011). "Thermostabilization of Bacillus circulans xylanase: computational optimization of unstable residues based on thermal fluctuation analysis." J Biotechnol **151**(1): 56-65.
Joo, J. C., S. Pohkrel, S. P. Pack and Y. J. Yoo (2010). "Thermostabilization of Bacillus circulans xylanase via computational design of a flexible surface cavity." J Biotechnol. **146**(1-2): 31-39.

Juge, N., M. L. Gal-Coeffet, C. Furniss, A. Gunning, B. Kramhoft, V. J. Morris, G. Williamson and B. Svensson (2002). "The starch binding domain of glucoamylase from Aspergillus niger: overview of its structure, function, and role in raw-starch hydrolysis." Biologia, Bratisl. **57**: 239-245
Juhasz, T., Z. Szengyel, K. Reczey, M. Siika-Aho and L. Viikari (2005). "Characterization of cellulases and hemicellulases produced by Trichoderma reesei on various carbon sources." Process Biochem **40**: 3519 - 3525.
Jun, H., H. Guangye and C. Daiwen (2013b). "Insights into enzyme secretion by filamentous fungi: comparative proteome analysis of Trichoderma reesei grown on different carbon sources." J Proteomics. **89**: 191-201.
Jung, K. H., J.-H. Lee, Y.-T. Yi, H.-K. Kim and M.-Y. Pack (1992). "Properties of a novel Clostridium thermocellum endo-β-1,4-glucanase expressed in Escherichia coli." Kor. J. Appl. Microbiol. Biotechnol **20**: 505-510.
Jürgens, C., A. Strom, D. Wegener, S. Hettwer, M. Wilmanns and R. Sterner (2000). "Directed evolution of a (βα)8-barrel enzyme to catalyze related reactions in two different metabolic pathways." Proceedings of the National Academy of Sciences **97**(18): 9925-9930.
Juturu, V., T. M. Teh and J. C. Wu (2014). "Expression of Aeromonas punctata ME-1 exo-xylanase X in E. coli for efficient hydrolysis of xylan to xylose." Applied Biochemistry and Biotechnology **174**(8): 2653-2662.
Kakiuchi, M., A. Isui, K. Suzuki, T. Fujino, E. Fujino, T. Kimura, S. Karita, K. Sakka and K. Ohmiya (1998). "Cloning and DNA sequencing of the genes encoding Clostridium josui scaffolding protein CipA and cellulase CelD and identification of their gene products as major components of the cellulosome." J Bacteriol. **180**(16): 4303-4308.
Kamat, R. K., W. Ma, Y. Yang, Y. Zhang, C. Wang, C. V. Kumar and Y. Lin (2013). "Adsorption and hydrolytic activity of the polycatalytic cellulase nanocomplex on cellulose." ACS Appl Mater Interfaces **5**(17): 8486-8494.
Kapich, A. N., T. V. Korneichik, A. Hatakka and K. E. Hammel (2010). "Oxidizability of unsaturated fatty acids and of a non-phenolic lignin structure in the manganese peroxidase-dependent lipid peroxidation system." Enzyme and Microbial Technology **46**: 136-140.
Kapich, A. N., K. T. Steffen, M. Hofrichter and A. Hatakka (2005). "Involvement of lipid peroxidation in the degradation of a non-phenolic lignin model compound by manganese peroxidase of the litter-decomposing fungus Stropharia coronilla." Biochemical and Biophysical Research Communications **330**(2): 371-377.
Karlsson, J., M. Siika-aho, M. Tenkanen and F. Tjerneld (2002). "Enzymatic properties of the low molecular mass endoglucanases Cel12A (EG III) and Cel45A (EG V) of Trichoderma reesei." J Biotechnol **99**: 63-78.
Karpol, A., Y. Barak, R. Lamed, Y. Shoham and E. A. Bayer (2008). "Functional asymmetry in cohesin binding belies inherent symmetry of the dockerin module: insight into cellulosome assembly revealed by systematic mutagenesis." Biochem J **410**(2): 331-338.
Kataeva, I., X.-L. Li, H. Chen, S.-K. Choi and L. G. Ljungdahl (1999). "Cloning and sequence analysis of a new cellulase gene encoding CelK, a major cellulosome component of Clostridium thermocellum: evidence for gene duplication and recombination." Journal of Bacteriology **181**(17): 5288-5295.

Kataria, R. and S. Ghosh (2011). "Saccharification of Kans grass using enzyme mixture from Trichoderma reesei for bioethanol production." Bioresour Technol. **102**(21): 9970-9975.
Katrolia, P., Q. Yan, P. Zhang, P. Zhou, S. Yang and Z. Jiang (2013). "Gene cloning and enzymatic characterization of an alkali-tolerant endo-1,4-β-mannanase from Rhizomucor miehei." J Agric Food Chem. **61**(2): 394-401.
Kauffman, S. A. (1993). The origins of order: Self organization and selection in evolution. New York, Oxford University Press.
Kawai, S., T. Umezawa and T. Higuchi (1988). "Degradation mechanisms of phenolic β-1 lignin substructure model compounds by laccase of Coriolus versicolor." Archives of Biochemistry and Biophysics **262**(1): 99-110.
Kazlauskas, R. J. and U. T. Bornscheuer (2009). "Finding better protein engineering strategies." Nat Chem Biol **5**(8): 526-569.
Kelley, R. L. and C. A. Reddy (1986). "Identification of glucose oxidase activity as the primary source of hydrogen peroxide production in ligninolytic cultures of Phanerochaete chrysosporium." Archives of Microbiology **144**(3): 248-253.
Kempton, J. B. and S. G. Withers (1992). "Mechanism of Agrobacterium β-glucosidase: kinetic studies." Biochemistry **31**: 9961-9969
Kersten, P. J. (1990). "Glyoxal oxidase of Phanerochaete chrysosporium: its characterization and activation by lignin peroxidase." Proc Natl Acad Sci U S A **87**(8): 2936-2940.
Kersten, P. J. and T. K. Kirk (1987). "Involvement of a new enzyme, glyoxal oxidase, in extracellular H2O2 production by Phanerochaete chrysosporium." Journal of Bacteriology **169**(5): 2195-2201.
Kester, H. C., D. agaud, C. Roy, D. Anker, A. Doutheau, V. Shevchik, N. Hugouvieux-Cotte-Pattat, J. A. Benen and J. Visser (1999a). "Performance of selected microbial pectinases on synthetic monomethyl-esterified di- and trigalacturonates." J Biol Chem. **274**(52): 37053-37059.
Kester, H. C. M., J. A. E. Benen and J. Visser (1999b). "The exopolygalacturonase from Aspergillus tubingensis is also active on xylogalacturonan." Biotechnol. Appl. Biochem. **30**: 53-57.
Khalili, B., F. Nourbakhsh, N. Nili, H. Khademi and B. Sharifna (2011). "Diversity of soil cellulase isoenzymes is associated with soil cellulase kinetic and thermodynamic parameters." Soil Biology and Biochemistry **43**(8): 1639-1648.
Kim, J., S. Kim, S. Yoon, E. Hong and Y. Ryu (2015b). "Improved enantioselectivity of thermostable esterase from Archaeoglobus fulgidus toward (S)-ketoprofen ethyl ester by directed evolution and characterization of mutant esterases." Appl Microbiol Biotechnol. **99**(15): 6293-6301.
Kim, S. J., K. Ishikawa, M. Hirai and M. Shoda (1995). "Characteristics of a newly isolated fungus, Geotrichum candidum Dec 1, which decolorizes various dyes." J. Ferment. Bioeng. **79**(6): 601-607.
Kim, S. J. and M. Shoda (1999). "Purification and characterization of a novel peroxidase from Geotrichum candidum dec 1 involved in decolorization of dyes." Appl Environ Microbiol. **65**(3): 1029-1035.
Kim, Y.-S., H.-C. Jung and J.-G. Pan (2000). "Bacterial cell surface display of an enzyme library for selective screening of improved cellulase variants." Applied and Environmental Microbiology **66**(2): 788-793.

Kirby, R. (2006). "Actinomycetes and lignin degradation." Adv Appl Microbiol **58**: 125-168.
Kirikyali, N. and I. F. Connerton (2014). "Heterologous expression and kinetic characterisation of Neurospora crassa β-xylosidase in Pichia pastoris." Enzyme and Microbial Technology **57**: 63-68.
Klein-Marcuschamer, D., P. Oleskowicz-Popiel, B. Simmons and H. Blanch (2012). "The challenge of enzyme cost in the production of lignocellulosic biofuels." Biotechnol Bioeng **109**(4): 1083-1087.
Kleman-Leyer, K. M., M. Siika-Aho, T. T. Teeri and T. K. Kirk (1996). "The cellulases endoglucanase I and cellobiohydrolase II of Trichoderma reesei act synergistically to solubilize native cotton cellulose but not to decrease its molecular size." Appl Environ Microbiol. **62**(8): 2883-2887.
Knowles, J., P. Lehtovaara and T. Teeri (1987). "Cellulase families and their genes " Trends Biotechnol. **5**: 255-261.
Kobayashi, T., M. P. M. Romaniec, P. J. Barker, U. T. Gerngross and A. L. Demain (1993). "Nucleotide sequence of gene celM encoding a new endoglucanase (CelM) of Clostridium thermocellum and purification of the enzyme." J. Ferm. Bioeng. **76**(4): 251-256.
Kofod, L. V., S. Kauppinen, S. Christgau, L. N. Andersen, H. P. Heldt-Hansen, K. Dörreich and H. Dalbøge (1994). "Cloning and characterization of two structurally and functionally divergent rhamnogalacturonases from Aspergillus aculeatus." Journal of Biological Chemistry **269**(46): 29182-29189.
Kohring, S., J. Wiegel and F. Mayer (1990). "Subunit composition and glycosidic activities of the cellulase complex from Clostridium thermocellum JW20." Applied and Environmental Microbiology **56**(12): 3798-3804.
Koivula, A., T. Kinnari, V. Harjunpaa, L. Ruohonen, A. Teleman, T. Drakenberg, J. Rouvinen, T. A. Jones and T. T. Teeri (1998). "Tryptophan 272: an essential determinant of crystalline cellulose degradation by Trichoderma reesei cellobiohydrolase Cel6A." FEBS Lett **429**(3): 341-346.
Koksharov, M. I. and N. N. Ugarova (2011). "Thermostabilization of firefly luciferase by in vivo directed evolution." Protein Engineering Design and Selection **24**(11): 835-844.
Komor, R. S., P. A. Romero, C. B. Xie and F. H. Arnold (2012). "Highly thermostable fungal cellobiohydrolase I (Cel7A) engineered using predictive methods." Protein Engineering Design and Selection **25**(12): 827-833.
Kormelink, F. J. M., M. J. F. Searle-Van Leeuwen, T. M. Wood and A. G. J. Voragen (1991). "(1,4)-β-d-Arabinoxylan arabinofuranohydrolase: a novel enzyme in the bioconversion of arabinoxylan." Applied Microbiology and Biotechnology **35**(2): 231-232.
Koshland, D. E. (1953). "Stereochemestry and the mechanism of enzymatic reactions." Biological Reviews **28**(4): 416-436.
Kostylev, M. and D. B. Wilson (2012). "Synergistic interactions in cellulose hydrolysis." Biofuels **3**(1): 61-70.
Kosugi, A., K. Murashima and R. H. Doi (2002). "Xylanase and acetyl xylan esterase activities of XynA, a key subunit of the Clostridium cellulovorans cellulosome for xylan degradation." Applied and Environmental Microbiology **68**(12): 6399-6402.
Kothari, V., P. Surt and D. Kapadia (2011). "Cellulosomes - A robust machinery for cellulose degradation " IJLST **4**(5): 31-36
Kremer, S. M. and P. M. Wood (1992a). "Production of Fenton's reagent by cellobiose oxidase from cellulolytic cultures of Phanerochaete chrysosporium." Eur J Biochem **208**(3): 807-814.

Kremer, S. M. and P. M. Wood (1992b). "Evidence that cellobiose oxidase from Phanerochaete chrysosporium is primarily an Fe(III) reductase. Kinetic comparison with neutrophil NADPH oxidase and yeast flavocytochrome b2." Eur J Biochem. **205**(1): 133-138.
Kristufek, D., S. Zeilinger and C. P. Kubicek (1995). "Regulation of β-xylosidase formation by xylose in Trichoderma reesei." Applied Microbiology and Biotechnology **42**(5): 713-717.
Kruus, K., A. C. Lua, A. L. Demain and J. H. Wu (1995). "The anchorage function of CipA (CelL), a scaffolding protein of the Clostridium thermocellum cellulosome." Proceedings of the National Academy of Sciences **92**(20): 9254-9258.
Kubata, B. K., T. Suzuki, H. Horitsu, K. Kawai and K. Takamizawa (1994). "Purification and characterization of Aeromonas caviae ME-1 xylanase V, which produces exclusively xylobiose from xylan." Appl Environ Microbiol **60**(2): 531-535.
Kubata, B. K., K. Takamizawa, K. Kawai, T. Suzuki and H. Horitsu (1995). "Xylanase IV, an exoxylanase of Aeromonas caviae ME-1 which produces xylotetraose as the only low-molecular-weight oligosaccharide from xylan." Appl Environ Microbiol **61**(4): 1666-1668.
Kuhls, K., E. Lieckfeldt, G. J. Samuels, W. Kovacs, W. Meyer, O. Petrini, W. Gams, T. Börner and C. P. Kubicek (1996). "Molecular evidence that the asexual industrial fungus Trichoderma reesei is a clonal derivative of the ascomycete Hypocrea jecorina." Proc Natl Acad Sci U S A **93**(15): 7755-7760.
Kuipers, R. K., H. J. Joosten, W. J. van Berkel, N. G. Leferink, E. Rooijen, E. Ittmann, F. van Zimmeren, H. Jochens, U. Bornscheuer, G. Vriend, V. A. dos Santos and P. J. Schaap (2010). "3DM: systematic analysis of heterogeneous superfamily data to discover protein functionalities." Proteins **78**(9): 2101-2113.
Kumar, L., G. Awasthi and B. Singh (2011b). "Extremophiles: a novel source of industrially important enzymes." Biotechnology **10**(2): 121-135.
Kuwahara, M., J. K. Glenn, M. A. Morgan and M. H. Gold (1984). "Separation and characterization of two extracelluar H2O2 -dependent oxidases from ligninolytic cultures of Phanerochaete chrysosporium." FEBS Letters **169**(2): 247-250.
Lamed, R., E. Setter and E. A. Bayer (1983a). "Characterization of a cellulose-binding, cellulase-containing complex in Clostridium thermocellum." J Bacteriol **156**(2): 828-836.
Lamed, R., E. Setter, R. Kenig and E. A. Bayer (1983b). "The cellulosome: a discrete cell surface organelle of Clostridium thermocellum which exhibits separate antigenic, cellulose-binding and various cellulolytic activities." Biotechnol. Bioeng. Symp **13**: 163-181
Langston, J., T. Shaghasi, E. Abbate, F. Xu, E. Vlasenko and M. Sweeney (2011). "Oxidoreductive cellulose depolymerization by the enzymes cellobiose dehydrogenase and glycoside hydrolase 61." Appl Environ Microbiol **77**: 7007 - 7015.
Larsbrink, J., A. Izumi, F. M. Ibatullin, A. Nakhai, H. J. Gilbert, G. J. Davies and H. Brumer (2011). "Structural and enzymatic characterization of a glycoside hydrolase family 31 α-xylosidase from Cellvibrio japonicus involved in xyloglucan saccharification." Biochem J. **436**(3): 567-580.
Lebbink, J. H. G., T. Kaper, P. Bron, J. van der Oost and W. M. de Vos (2000). "Improving low-temperature catalysis in the hyperthermostable Pyrococcus furiosus β-glucosidase CelB by directed evolution." Biochemistry **39**(13): 3656-3665.
Lee, C. C., R. E. Kibblewhite, K. Wagschal, R. Li, G. H. Robertson and W. J. Orts (2012). "Isolation and characterization of a novel GH67 α-glucuronidase from a mixed culture." J Ind Microbiol Biotechnol. **39**(8): 1245-1251.

Lee, H. L., C. K. Chang, W. Y. Jeng, A. H. Wang and P. H. Liang (2012). "Mutations in the substrate entrance region of β-glucosidase from Trichoderma reesei improve enzyme activity and thermostability." **25**: 33-40.
Lee, R. C., R. A. Burton, M. Hrmova and G. B. Fincher (2001). "Barley arabinoxylan arabinofuranohydrolases: purification, characterization and determination of primary structures from cDNA clones." Biochem J **356**(Pt 1): 181-189.
Lee, R. C., M. Hrmova, R. A. Burton, J. Lahnstein and G. B. Fincher (2003a). "Bifunctional family 3 glycoside hydrolases from barley with alpha -L-arabinofuranosidase and beta -D-xylosidase activity. Characterization, primary structures, and COOH-terminal processing." J Biol Chem **278**(7): 5377-5387.
Lehmann, C., M. Bocola, W. R. Streit, R. Martinez and U. Schwaneberg (2014). "Ionic liquid and deep eutectic solvent-activated CelA2 variants generated by directed evolution." Appl Microbiol Biotechnol. **98**(12): 5775-5785.
Leibovitz, E. and P. Béguin (1996). "A new type of cohesin domain that specifically binds the dockerin domain of the Clostridium thermocellum cellulosome-integrating protein CipA." J. Bacteriol. **178**(11): 3077-3084.
Leibovitz, E., H. Ohayon, P. Gounon and P. Béguin (1997). "Characterization and subcellular localization of the Clostridium thermocellum scaffoldin dockerin binding protein SdbA." Journal of Bacteriology **179**(8): 2519-2523.
Leisola, M. S. A., B. Schmidt, U. Thanei-Wyss and A. Fiechter (1985). "Aromatic ring cleavage of veratryl alcohol by Phanerochaete chrysosporium." FEBS Lett. **189**(2): 267-270.
Lemaire, M., H. Ohayon, P. Gounon, T. Fujino and P. Béguin (1995). "OlpB, a new outer layer protein of Clostridium thermocellum, and binding of its S-layer-like domains to components of the cell envelope." Journal of Bacteriology **177**(9): 2451-2459.
Léonard, R., M. Pabst, J. S. Bondili, G. Chambat, C. Veit, R. Strasser and F. Altmann (2008). "Identification of an Arabidopsis gene encoding a GH95 alpha1,2-fucosidase active on xyloglucan oligo- and polysaccharides." Phytochemistry **69**(10): 1983-1988.
Leonowicz, A., K. Grzywnowicz and M. Malinowska (1979). "Oxidative and demethylating activity of multiple forms of laccase from Pholiota mutabilis." Acta Biochim Pol **26**(4): 431-434.
Leung, D. W., E. Chen and D. V. Goeddel (1989). "A method for random mutagenesis of a defined DNA segment using a modified polymerase chain reaction." Technique **1**: 11-15
Li, K., P. Azadi, R. Collins, J. Tolan, J. S. Kim and K. E. L. Eriksson (2000). "Relationships between activities of xylanases and xylan structures." Enzyme and Microbial Technology **27**(1-2): 89-94.
Li, X.-L., C. D. Skory, M. A. Cotta, V. Puchart and P. Biely (2008b). "Novel family of carbohydrate esterases, based on identification of the Hypocrea jecorina acetyl esterase gene." Applied and Environmental Microbiology **74**(24): 7482-7489.
Li, X.-L., S. Špániková, R. P. de Vries and P. Biely (2007b). "Identification of genes encoding microbial glucuronoyl esterases." FEBS Letters **581**(21): 4029-4035.
Li, X., W. T. Beeson, C. M. Phillips, M. A. Marletta and J. H. D. Cate (2012). "Structural basis for substrate targeting and catalysis by fungal polysaccharide monooxygenases." Structure **20**(6): 1051-1061.
Li, X. L., H. Chen and L. G. Ljungdahl (1997). "Two cellulases, CelA and CelC, from the polycentric anaerobic fungus Orpinomyces strain PC-2 contain N-terminal docking domains for a cellulase-hemicellulase complex." Appl. Environ. Microbiol. **63**(12): 4721-4728.

Li, Y., D. C. Irwin and D. B. Wilson (2007a). "Processivity, substrate binding, and mechanism of cellulose hydrolysis by Thermobifida fusca Cel9A." Appl Environ Microbiol. **73**(10): 3165-3172.
Liang, C., M. Fioroni, F. Rodriguez-Ropero, Y. Xue, U. Schwaneberg and Y. Ma (2011). "Directed evolution of a thermophilic endoglucanase (Cel5A) into highly active Cel5A variants with an expanded temperature profile." J Biotechnol **154**(1): 46-53.
Liebgott, P. P., A. L. de Lacey, B. Burlat, L. Cournac, P. Richaud, M. Brugna, V. M. Fernandez, B. Guigliarelli, M. Rousset, C. Léger and S. Dementin (2010). "Original design of an oxygen-tolerant [NiFe] hydrogenase: major effect of a valine-to-cysteine mutation near the active site." J Am Chem Soc. **133**(4): 986-997.
Liers, C., C. Bobeth, M. Pecyna, R. Ullrich and M. Hofrichter (2010). "DyP-like peroxidases of the jelly fungus Auricularia auricula-judae oxidize nonphenolic lignin model compounds and high-redox potential dyes." Applied Microbiology and Biotechnology **85**(6): 1869-1879.
Lin, L., X. Meng, P. Liu, Y. Hong, G. Wu, X. Huang, C. Li, J. Dong, L. Xiao and Z. Liu (2009). "Improved catalytic efficiency of endo-beta-1,4-glucanase from Bacillus subtilis BME-15 by directed evolution." Appl Microbiol Biotechnol **82**(4): 671-679.
Liu, H., L. Zhu, M. Bocola, N. Chen, A. C. Spiess and U. Schwaneberg (2013b). "Directed laccase evolution for improved ionic liquid resistance." Green Chemistry **15**(5): 1348-1355.
Liu, M., J. Gu, W. Xie and H. Yu (2013a). "Directed co-evolution of an endoglucanase and a β-glucosidase in Escherichia coli by a novel high-throughput screening method." Chem. Commun. **49**: 7219-7221.
Liu, W., X. Z. Zhang, Z. Zhang and Y. H. Zhang (2010). "Engineering of Clostridium phytofermentans endoglucanase Cel5A for improved thermostability." Appl Environ Microbiol **76**(14): 4914-4917.
Liu, Y.-S., J. Baker, Y. Zeng, M. Himmel, T. Haas and S.-Y. Ding (2011). "Cellobiohydrolase hydrolyzes crystalline cellulose on hydrophobic faces." The journal of biological chemestry **286**(13): 11195-11201.
Lombard, V., H. Golaconda Ramulu, E. Drula, P. M. Couthino and B. Henrissat (2014). "The carbohydrate-active enzymes database (CAZy) in 2013." Nucleic Acids Res. **42**(D1): D490-D495.
Lunetta, J. M. and D. Pappagianis (2014). "Identification, molecular characterization, and expression analysis of a DOMON-like type 9 carbohydrate-binding module domain-containing protein of Coccidioides posadasii." Med Mycol **52**(6): 591-609.
Lynd, L., P. Weimer, W. van Zyl and I. Pretorius (2002). "Microbial cellulose utilization: Fundamentals and biotechnology." Microbiol Mol Biol R **66**: 506 - +.
Lynd, L. R., P. J. Weimer, W. H. van Zyl and I. S. Pretorius (2002). "Microbial cellulose utilization: fundamentals and biotechnology." Microbiology and Molecular Biology Reviews **66**(3): 506-577.
Lytle, B. L., B. F. Volkman, W. M. Westler, M. P. Heckman and J. H. Wu (2001). "Solution structure of a type I dockerin domain, a novel prokaryotic, extracellular calcium-binding domain." J Mol Biol **307**(3): 745-753.
Lytle, B. L., B. F. Volkman, W. M. Westler and J. H. D. Wu (2000). "Secondary structure and calcium-induced folding of the Clostridium thermocellum dockerin domain determined by NMR spectroscopy." Archives of Biochemistry and Biophysics **379**(2): 237-244.
Lytle, B. L., W. M. Westler and J. H. D. Wu (1999). Molecular assembly of the Clostridium thermocellum cellulosome. Genetics, biochemistry, and ecology of cellulose degradation. K. Ohmiya, K. Hayashi, K. Sakka et al. Tokyo, Japan, Uni Publishers**:** 444–449.

Mai, V., J. Wiegel and W. W. Lorenz (2000). "Cloning, sequencing, and characterization of the bifunctional xylosidase-arabinosidase from the anaerobic thermophile thermoanaerobacter ethanolicus." Gene **247**(1-2): 137-143.
Malarczyk, E., J. Kochmanska-Rdest and A. Jarosz-Wilkolazka (2009). "Influence of very low doses of mediators on fungal laccase activity - nonlinearity beyond imagination." Nonlinear Biomedical Physics **3**(10).
Mandels, M. and E. T. Reese (1999). "Fungal cellulases and the microbial decomposition of cellulosic fabric." Journal of Industrial Microbiology and Biotechnology **5**(4-5): 225-240.
Mandels, M. a. R., , E.T.Ann (1965). Rev. Phytopathol **3**: 85.
Margolles-Clark, E., M. Saloheimo, M. Siika-aho and M. Penttilä (1996b). "The α-glucuronidase-encoding gene of Trichoderma reesei." Gene **172**(1): 171-172.
Margolles-Clark, E., M. Tenkanen, E. Luonteri and M. Penttilä (1996d). "Three α-Galactosidase Genes of Trichoderma reesei Cloned by Expression in Yeast." European Journal of Biochemistry **240**(1): 104-111.
Margolles-Clark, E., M. Tenkanen, T. Nakari-Setälä and M. Penttilä (1996c). "Cloning of genes encoding alpha-L-arabinofuranosidase and beta-xylosidase from Trichoderma reesei by expression in Saccharomyces cerevisiae." Applied and Environmental Microbiology **62**(10): 3840-3846.
Margolles-Clark, E., M. Tenkanen, H. Söderlund and M. Penttilä (1996a). "Acetyl xylan esterase from Trichoderma reesei contains an active-site serine residue and a cellulose-binding domain." European Journal of Biochemistry **237**(3): 553-560.
Markovic, O. and S. Janecek (2001). "Pectin degrading glycoside hydrolases of family 28: sequence-structural features, specificities and evolution." Protein Eng. **14**(9): 615-631.
Martinez, D., R. Berka, B. Henrissat, M. Saloheimo, M. Arvas, S. Baker, J. Chapman, O. Chertkov, P. Coutinho and D. Cullen (2008). "Genome sequencing and analysis of the biomass-degrading fungus Trichoderma reesei (syn. Hypocreajecorina)." Nat Biotechnol **26**(5): 553 - 560.
Martinez, D., R. M. Berka, B. Henrissat, M. Saloheimo, M. Arvas, S. E. Baker, J. Chapman, O. Chertkov, P. M. Coutinho, D. Cullen, E. G. J. Danchin, I. V. Grigoriev, P. Harris, M. Jackson, C. P. Kubicek, C. S. Han, I. Ho, L. F. Larrondo, A. L. de Leon, J. K. Magnuson, S. Merino, M. Misra, B. Nelson, N. Putnam, B. Robbertse, A. A. Salamov, M. Schmoll, A. Terry, N. Thayer, A. Westerholm-Parvinen, C. L. Schoch, J. Yao, R. Barabote, M. A. Nelson, C. Detter, D. Bruce, C. R. Kuske, G. Xie, P. Richardson, D. S. Rokhsar, S. M. Lucas, E. M. Rubin, N. Dunn-Coleman, M. Ward and T. S. Brettin (2008). "Genome sequencing and analysis of the biomass-degrading fungus Trichoderma reesei (syn. Hypocrea jecorina)." Nat Biotech **26**(5): 553-560.
Martinez, M. J., B. Böckle, S. Camarero, F. Guillén and A. T. Martinez (1996). MnP isoenzymes produced by two Pleurotus species in liquid culture and during wheat-straw solid-state fermentation. Enzymes for Pulp and Paper Processing, American Chemical Society. **655:** 183-196.
Martinez, M. J., F. J. Ruiz-Duenas, F. Guillen and A. T. Martinez (1996). "Purification and catalytic properties of two manganese peroxidase isoenzymes from Pleurotus eryngii." Eur J Biochem **237**(2): 424-432.
Mateo, C., J. M. Palomo, G. Fernandez-Lorente, J. M. Guisan and R. Fernandez-Lafuente (2007). "Improvement of enzyme activity, stability and selectivity via immobilization techniques." Enzyme and Microbial Technology **40**(6): 1451-1463.

Matsumura, I. (2000). Accelerating the discovery, modification and commercialization of enzyme applications IBC's Fifth Annual World Congress on Enzyme Technologies. Las Vegas.
Matsuo, M. and T. Yasui (1984). "Purification and some properties of beta-xylosidase from Trichoderma viride." Agricultural and biological chemistry **48**(7): 1845-1852.
Matte, A. and C. W. Forsberg (1992). "Purification, characterization, and mode of action of endoxylanases 1 and 2 from Fibrobacter succinogenes S85." Appl Environ Microbiol **58**(1): 157-168.
Maurya, D. P., D. Singh, D. Pratap and J. P. Maurya (2012). "Optimization of solid state fermentation conditions for the production of cellulase by Trichoderma reesei." J Environ Biol. **33**(1): 5-8.
May, O., P. T. Nguyen and F. H. Arnold (2000). "Inverting enantioselectivity by directed evolution of hydantoinase for improved production of L-methionine." Nat Biotechnol **18**(3): 317-320.
Mayans, O., M. Scott, I. Connerton, T. Gravesen, J. Benen, J. Visser, R. Pickersgill and J. Jenkins (1997). "Two crystal structures of pectin lyase A from Aspergillus reveal a pH driven conformational change and striking divergence in the substrate-binding clefts of pectin and pectate lyases." Structure **5**(5): 677-689.
Mayer, F., M. P. Coughlan, Y. Mori and L. G. Ljungdahl (1987). "Macromolecular organization of the cellulolytic enzyme complex of Clostridium thermocellum as revealed by electron microscopy." Appl Environ Microbiol **53**(12): 2785-2792.
McCarthy, J. K., A. Uzelac, D. F. Davis and D. E. Eveleigh (2004). "Improved catalytic efficiency and active Sste modification of 1,4-β-d-glucan glucohydrolase a from Thermotoga neapolitana by directed evolution." Journal of Biological Chemistry **279**(12): 11495-11502.
Mel'nik, M. S., D. V. Kapkov, M. A. Mogutov, M. L. Rabinovich and A. A. Klesov (1989). "A new type of Clostridium thermocellum endoglucanase produced by the recombinant strain of E. coli. Some properties and identification in donor cells." Biokhimiia **54**: 387-395.
Mel'nik, M. S., M. L. Rabinovich and Y. V. Voznyi (1991). "Cellobiohydrolase of Clostridium thermocellum produced by a recombinant E. coli strain." Biokhimiya **56**: 1787-1797.
Mello, B. L. and I. Polikarpov (2014). "Family 1 carbohydrate binding-modules enhance saccharification rates." AMB Express. **4**(36).
Melzer, S., C. Sonnendecker, C. Föllner and W. Zimmermann (2015). "Stepwise error-prone PCR and DNA shuffling changed the pH activity range and product specificity of the cyclodextrin glucanotransferase from an alkaliphilic Bacillus sp." FEBS Open Bio **5**: 528-534.
Mercer, D. K., M. Iqbal, P. Miller and A. J. McCarthy (1996). "Screening actinomycetes for extracellular peroxidase activity." Appl Environ Microbiol **62**(6): 2186-2190.
Merino, S. and J. Cherry (2007). Progress and Challenges in Enzyme Development for Biomass Utilization. Biofuels. L. Olsson, Springer Berlin Heidelberg. **108:** 95-120.
Merino, S. T. and J. Cherry (2007). Progress and challenges in enzyme development for biomass utilization. Advances in Biochemical Engineering/Biotechnology. T. Scheper, S. Belkin, P. M. Doran et al., Springer. **108:** 95-120.
Messerschmidt, A. (1997). Multi-copper oxidases. Singapore, World Scientific Pub Co Inc.
Mester, T., K. Ambert-Balay, S. Ciofi-Baffoni, L. Banci, A. D. Jones and M. Tien (2001). "Oxidation of a tetrameric nonphenolic lignin model compound by lignin peroxidase." J Biol Chem. **276**(25): 22985-22990.

Metz, B., V. Seidl-Seiboth, T. Haarmann, A. Kopchinskiy, P. Lorenz, B. Seiboth and C. P. Kubicek (2011). "Expression of biomass-degrading enzymes is a major event during conidium development in Trichoderma reesei." Eukaryotic Cell **10**(11): 1527-1535.
Mihoc, A. and D. Kluepfel (1990). "Purification and characterization of a β-glucosidase from Streptomyces lividans 66." Canadian Journal of Microbiology **36**(1): 53-56.
Millis, C. D., D. Cai, M. T. Stankovich and M. Tien (1989). "Oxidation-reduction potentials and ionization states of extracellular peroxidases from the lignin-degrading fungus Phanerochaete chrysosporium." Biochemistry **28**(21): 8484-8489.
Mills, D. R., R. L. Peterson and S. Spiegelman (1967). "An extracellular Darwinian experiment with a self-duplicating nucleic acid molecule." Proc Natl Acad Sci U S A **58**(1): 217-224.
Miras, I., F. Schaeffer, P. Béguin and P. M. Alzari (2002). "Mapping by site-directed mutagenesis of the region responsible for cohesin−dockerin interaction on the surface of the seventh cohesin domain of Clostridium thermocellum CipA." Biochemistry **41**(7): 2115-2119.
Mohamed, S. A., N. M. Farid, E. N. Hossiny and R. I. Bassuiny (2006). "Biochemical characterization of an extracellular polygalacturonase from Trichoderma harzianum." Journal of Biotechnology **127**(1): 54-64.
Mohanram, S., D. Amat, J. Choudhary, A. Arora and L. Nain (2013). "Novel perspectives for evolving enzyme cocktails for lignocellulose hydrolysis in biorefineries." Sustainable Chemical Processes **1**(15).
Moore, J. C. and F. H. Arnold (1996). "Directed evolution of a para-nitrobenzyl esterase for aqueous-organic solvents." Nat Biotechnol **14**(4): 458-467.
Morag, E., E. A. Bayer and R. Lamed (1990). "Relationship of cellulosomal and noncellulosomal xylanases of Clostridium thermocellum to cellulose-degrading enzymes." Journal of Bacteriology **172**(10): 6098-6105.
Morag, E., A. Lapidot, D. Govorko, R. Lamed, M. Wilchek, E. A. Bayer and Y. Shoham (1995). "Expression, purification, and characterization of the cellulose-binding domain of the scaffoldin subunit from the cellulosome of Clostridium thermocellum." Appl. Environ. Microbiol. **61**(5): 1980-1986.
Moran, F., S. Nasuno and M. P. Starr (1968). "Extracellular and intracellular polygllacturonic acid trans-eliminases of Erwinia carotovora." Arch Biochem Biophys. **123**(2): 298-306.
Moreira, L. R. S. and E. X. F. Filho (2008). "An overview of mannan structure and mannan-degrading enzyme systems." Applied Microbiology and Biotechnology **79**(2): 165-178.
Morley, K. L. and R. J. Kazlauskas (2005). "Improving enzyme properties: when are closer mutations better?" Trends Biotechnol **23**(5): 231-237.
Morpeth, F. F. (1985). "Some properties of cellobiose oxidase from the white-rot fungus Sporotrichum pulverulentum." Biochem J. **228**(3): 557-564.
Moser, F., D. Irwin, S. Chen and D. Wilson (2008). "Regulation and characterization of Thermobifida fusca carbohydrate-binding module proteins E7 and E8." Biotechnol Bioeng **100**: 1066 - 1077.
Mosolova, T. P., S. V. Kalyuzhnyi, S. D. Varfolomeyev and G. A. Velikodvorskaya (1993). "Purification and properties of Clostridium thermocellum endoglucanase 5 produced inEscherichia coli." Applied Biochemistry and Biotechnology **42**(1): 9-18.

Muheim, A., R. Waldner, D. Sanglard, J. Reiser, H. E. Schoemaker and M. S. Leisola (1991). "Purification and properties of an aryl-alcohol dehydrogenase from the white-rot fungus Phanerochaete chrysosporium." Eur. J. Biochem. **195**(2): 369-375.
Muller, D. (1936). "Glucose oxidase." Ergebn. Enzymforsch **5**: 259.
Müller, D. (1928). "Studies on the new enzyme glucose oxidase. I." Biochem. Z. **199**: 136-170.
Murashima, K., A. Kosugi and R. H. Doi (2002b). "Thermostabilization of cellulosomal endoglucanase EngB from Clostridium cellulovorans by in vitro DNA recombination with non-cellulosomal endoglucanase EngD." Molecular Microbiology **45**(3): 617-626.
Mutter, M., G. Beldman, S. M. Pitson, H. A. Schols and A. G. Voragen (1998a). "Rhamnogalacturonan α-D-galactopyranosyluronohydrolase. An enzyme that specifically removes the terminal nonreducing galacturonosyl residue in rhamnogalacturonan regions of pectin." Plant Physiol. **117**: 153-163.
Mutter, M., G. Beldman, H. A. Schols and A. G. Voragen (1994). "Rhamnogalacturonan alpha-L-rhamnopyranohydrolase. A novel enzyme specific for the terminal nonreducing rhamnosyl unit in rhamnogalacturonan regions of pectin." Plant Physiol **106**(1): 241-250.
Mutter, M., I. J. Colquhoun, G. Beldman, H. A. Schols, E. J. Bakx and A. G. Voragen (1998b). "Characterization of recombinant rhamnogalacturonan alpha-L-rhamnopyranosyl-(1,4)-alpha-D-galactopyranosyluronide lyase from Aspergillus aculeatus. An enzyme that fragments rhamnogalacturonan I regions of pectin." Plant Physiol **117**(1): 141-152.
Mutter, M., I. J. Colquhoun, H. A. Schols, G. Beldman and A. G. Voragen (1996). "Rhamnogalacturonase B from Aspergillus aculeatus is a rhamnogalacturonan alpha-L-rhamnopyranosyl-(1-->4)-alpha-D-galactopyranosyluronide lyase." Plant Physiol **110**(1): 73-77.
Nagy, T., R. B. Tunnicliffe, L. D. Higgins, C. Walters, H. J. Gilbert and M. Williamson (2007). "Characterization of a double dockerin from the cellulosome of the anaerobic fungus Piromyces equi." J Mol Biol. **373**(3): 612-622.
Nakazawa, H., K. Okada, T. Onodera, W. Ogasawara, H. Okada and Y. Morikawa (2009). "Directed evolution of endoglucanase III (Cel12A) from Trichoderma reesei." Appl Microbiol Biotechnol **83**(4): 649-657.
Navarro, D., M. Couturier, G. G. da Silva, J. G. Berrin, X. Rouau, M. Asther and C. Bignon (2010). "Automated assay for screening the enzymatic release of reducing sugars from micronized biomass." Microb Cell Fact **9**(58).
Ness, J. E., M. Welch, L. Giver, M. Bueno, J. R. Cherry, T. V. Borchert, W. P. Stemmer and J. Minshull (1999). "DNA shuffling of subgenomic sequences of subtilisin." Nat Biotechnol **17**(9): 893-896.
Ng, T.-K., L. R. Gahan, G. Schenk and D. L. Ollis (2015). "Altering the substrate specificity of methyl parathion hydrolase with directed evolution." Arch Biochem Biophys. **573**: 59-68.
Ng, T. K. and J. G. Zeikus (1981). "Purification and characterization of an endoglucanase (1, 4-beta-D-glucan glucanohydrolase) from Clostridium thermocellum." Biochemical Journal **199**(2): 341.
Notenboom, V., A. B. Boraston, P. Chiu, A. C. Freelove, D. G. Kilburn and D. R. Rose (2001). "Recognition of cello-oligosaccharides by a family 17 carbohydrate-binding module: an X-ray crystallographic, thermodynamic and mutagenic study." J Mol Biol **314**(4): 797-806.
Nousiainen, P., P. Maijala, A. Hatakka, A. T. Martínez and J. Sipilä (2009). "Syringyl-type simple plant phenolics as mediating oxidants in laccase catalyzed degradation of lignocellulosic

materials: Model compound studies 10th EWLP, Stockholm, Sweden, August 25–28, 2008." Holzforschung **63**(6): 699-704.
Nurizzo, D., T. Nagy, H. J. Gilbert and G. J. Davies (2002). "The structural basis for catalysis and specificity of the Pseudomonas cellulosa alpha-glucuronidase, GlcA67A." Structure **10**(4): 547-556.
Oh, K. H., S. H. Nam and H. S. Kim (2002). "Directed evolution of N-carbamyl-D-amino acid amidohydrolase for simultaneous improvement of oxidative and thermal stability." Biotechnol Prog **18**(3): 413-417.
Okada, H., K. Tada, T. Sekiya, K. Yokoyama, A. Takahashi, H. Tohda, H. Kumagai and Y. Morikawa (1998). "Molecular characterization and heterologous expression of the gene encoding a low-molecular-mass endoglucanase from Trichoderma reesei QM9414." Applied and Environmental Microbiology **64**(2): 555-563.
Oliveira, O. V., L. C. Freitas, T. P. Straatsma and R. D. Lins (2009). "Interaction between the CBM of Cel9A from Thermobifida fusca and cellulose fibers." J Mol Recognit. **22**(1): 38-45.
Olsen, M., B. Iverson and G. Georgiou (2000). "High-throughput screening of enzyme libraries." Current Opinion in Biotechnology **11**(4): 331-337.
Otten, L. G. and W. J. Quax (2005). "Directed evolution: selecting today's biocatalysts." Biomolecular Engineering **22**(1-3): 1-9.
Ouyang, J., M. Yan, D. Kong and L. Xu (2006). "A complete protein pattern of cellulose and hemicellulase genes in the filamentous fungus Trichoderma reesei." Biotechnol J **1**(11): 1266-1274.
Packer, M. S. and D. R. Liu (2015). "Methods for the directed evolution of proteins." Nat Rev Genet. **16**(7): 379-394.
Pagès, S., A. Bélaïch, J.-P. Bélaïch, E. Morag, R. Lamed, Y. Shoham and E. A. Bayer (1997). "Species-specificity of the cohesin-dockerin interaction between Clostridium thermocellum and Clostridium cellulolyticum: Prediction of specificity determinants of the dockerin domain." Proteins: Structure, Function, and Bioinformatics **29**(4): 517-527.
Pagès, S., A. Bélaïch, H.-P. Fierobe, C. Tardif, C. Gaudin and J.-P. Bélaïch (1999). "Sequence analysis of scaffolding protein CipC and ORFXp, a new cohesin-containing protein in Clostridium cellulolyticum: comparison of various cohesin domains and subcellular localization of ORFXp." Journal of Bacteriology **181**(6): 1801-1810.
Parsiegla, G., M. Juy, C. Reverbel-Leroy, C. Tardif, J. P. Belaich, H. Driguez and R. Haser (1998). "The crystal structure of the processive endocellulase CelF of Clostridium cellulolyticum in complex with a thiooligosaccharide inhibitor at 2.0 Å resolution." The EMBO Journal **17**(19): 5551-5562.
Paszczyński, A., V.-B. Huynh and R. Crawford (1986). "Comparison of ligninase-I and peroxidase-M2 from the white-rot fungus Phanerochaete chrysosporium." Archives of Biochemistry and Biophysics **244**(2): 750-765.
Patnaik, R., S. Louie, V. Gavrilovic, K. Perry, W. P. Stemmer, C. M. Ryan and S. del Cardayre (2002). "Genome shuffling of Lactobacillus for improved acid tolerance." Nat Biotechnol **20**(7): 707-712.
Pavelka, A., E. Chovancova and J. Damborsky (2009). "HotSpot Wizard: a web server for identification of hot spots in protein engineering." Nucleic Acids Res **37**(Web Server issue): W376-383.

Pedersen, M., K. S. Johansen and A. S. Meyer (2011). "Low temperature lignocellulose pretreatment: effects and interactions of pretreatment pH are critical for maximizing enzymatic monosaccharide yields from wheat straw." Biotechnology for Biofuels **4**(11): 3-10.
Pedrolli, D. B., A. C. Monteiro, E. Gomes and E. C. Carmona (2009). "Pectin and pectinases: production, characterization and industrial application of microbial pectinolytic enzymes." Open Biotechnol J **3**: 9-18.
Pei, X. Q., Z. L. Yi, C. G. Tang and Z. L. Wu (2011). "Three amino acid changes contribute markedly to the thermostability of beta-glucosidase BglC from Thermobifida fusca." Bioresour Technol **102**(3): 3337-3342.
Pell, G., M. P. Williamson, C. Walters, H. Du, H. J. Gilbert and D. N. Bolam (2003). "Importance of hydrophobic and polar residues in ligand binding in the family 15 carbohydrate-binding module from Cellvibrio japonicus Xyn10C." Biochemistry **42**(31): 9316-9323.
Peng, H., Y. Zheng, M. Chen, Y. Wang, Y. Xiao and Y. Gao (2014). "A starch-binding domain identified in α-amylase (AmyP) represents a new family of carbohydrate-binding modules that contribute to enzymatic hydrolysis of soluble starch." FEBS Lett. **588**(7): 1161-1167.
Penttilä, M., P. Lehtovaara, H. Nevalainen, R. Bhikhabhai and J. Knowles (1986). "Homology between cellulase genes of Trichoderma reesei: complete nucleotide sequence of the endoglucanase I gene." Gene **45**(3): 253-263.
Pérez-Boada, M., F. J. Ruiz-Duenas, R. Pogni, R. Basosi, T. Choinowski, M. J. Martínez, K. Piontek and A. T. Martínez (2005). "Versatile peroxidase oxidation of high redox potential aromatic compounds: site-directed mutagenesis, spectroscopic and crystallographic investigation of three long-range electron transfer pathways." Journal of molecular biology **354**(2): 385-402.
Perez, J. and T. W. Jeffries (1992). "Roles of manganese and organic acid chelators in regulating lignin degradation and biosynthesis of peroxidases by Phanerochaete chrysosporium." Appl Environ Microbiol **58**(8): 2402-2409.
Peters, M. W., P. Meinhold, A. Glieder and F. H. Arnold (2003). "Regio- and enantioselective alkane hydroxylation with engineered cytochromes P450 BM-3." J Am Chem Soc **125**(44): 13442-13450.
Pétré, D., J. Millet, R. Longin, P. Béguin, H. Girard and J.-P. Aubert (1986). "Purification and properties of the endoglucanase C of Clostridium thermocellum produced in Escherichia coli." Biochimie **68**(5): 687-695.
Petre, J., R. Longin and J. Millet (1981). "Purification and properties of an endo-β-1,4-glucanase from Clostridium thermocellum." Biochimie **63**(7): 629-639.
Phillips, C., W. Beeson, J. Cate and M. Marletta (2011). "Cellobiose dehydrogenase and a copper-dependent polysaccharide monooxygenase potentiate cellulose degradation by Neurospora crassa." ACS Chem Biol **6**: 1399 - 1406.
Phillips, C. M., W. T. Beeson, J. H. Cate and M. A. Marletta (2011). "Cellobiose dehydrogenase and a copper-dependent polysaccharide monooxygenase potentiate cellulose degradation by Neurospora crassa." ACS Chemical Biology **6**(12): 1399-1406.
Pickersgill, R., J. Jenkins, G. Harris, W. Nasser and J. Robert-Baudouy (1994). "The structure of Bacillus subtilis pectate lyase in complex with calcium." Nat Struct Biol **1**(10): 717-723.
Pinheiro, B. A., H. J. Gilbert, K. Sakka, K. Sakka, V. O. Fernandes, J. A. Prates, V. D. Alves, D. N. Bolam, L. M. Ferreira and C. M. Fontes (2009). "Functional insights into the role of novel type I cohesin and dockerin domains from Clostridium thermocellum." Biochem J **424**(3): 375-384.

Pleiss, J. (2012). Rational design of enzymes. Enzyme Catalysis in Organic Synthesis. K. Drauz, H. Gröger and O. May, Wiley-VCH**:** 89-117.
Pokhrel, S., J. C. Joo and Y. J. Yoo (2013). "Shifting the optimum pH of Bacillus circulans xylanase towards acidic side by introducing arginine." Biotechnology and Bioprocess Engineering **18**(1): 35-42.
Pokkuluri, P. R., N. E. C. Duke, S. J. Wood, M. A. Cotta, X.-L. Li, P. Biely and M. Schiffer (2011). "Structure of the catalytic domain of glucuronoyl esterase Cip2 from Hypocrea jecorina." Proteins: Structure, Function, and Bioinformatics **79**(8): 2588-2592.
Popp, J. L. and T. K. Kirk (1991). "Oxidation of methoxybenzenes by manganese peroxidase and by Mn^{3+}." Archives of Biochemistry and Biophysics **288**(1): 145-148.
Poutanen, K. (1988). "An alpha-L-arabinofuranosidase of Trichoderma reesei." J Biotechnol **7**: 271-282.
Qi, M., H. S. Jun and C. W. Forsberg (2007). "Characterization and synergistic interactions of Fibrobacter succinogenes glycoside hydrolases." Appl Environ Microbiol **73**(19): 6098-6105.
Quin, M. B. and C. Schmidt-Dannert (2011). "Engineering of biocatalysts - from evolution to creation." ACS Catal **1**(9): 1017-1021.
Quinlan, R. J., M. D. Sweeney, L. Lo Leggio, H. Otten, J. C. Poulsen, K. S. Johansen, K. B. Krogh, C. I. Jorgensen, M. Tovborg, A. Anthonsen, T. Tryfona, C. P. Walter, P. Dupree, F. Xu, G. J. Davies and P. H. Walton (2011). "Insights into the oxidative degradation of cellulose by a copper metalloenzyme that exploits biomass components." Proc. Natl. Acad. Sci. U S A **108**(37): 15079-15084.
Rabinovich, M. L., V. V. Nguen and A. A. Klesov (1982). "Adsorption of cellulolytic enzymes on cellulose and the kinetics of the adsorbed enzymes. Two modes for interaction of the enzymes with the insoluble substrate." Biokhimiia **47**(3): 465-477.
Raíces, M., R. Montesino, J. Cremata, B. García, W. Perdomo, I. Szabó, G. Henriksson, B. M. Hallberg, G. Pettersson and G. Johansson (2002). "Cellobiose quinone oxidoreductase from the white rot fungus Phanerochaete chrysosporium is produced by intracellular proteolysis of cellobiose dehydrogenase." Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression **1576**(1-2): 15-22.
Raillard, S., A. Krebber, Y. Chen, J. E. Ness, E. Bermudez, R. Trinidad, R. Fullem, C. Davis, M. Welch, J. Seffernick, L. P. Wackett, W. P. Stemmer and J. Minshull (2001). "Novel enzyme activities and functional plasticity revealed by recombining highly homologous enzymes." Chem Biol **8**(9): 891-898.
Raman, B., C. K. McKeown, M. Rodriguez, Jr., S. D. Brown and J. R. Mielenz (2011). "Transcriptomic analysis of Clostridium thermocellum ATCC 27405 cellulose fermentation." BMC Microbiol **11**: 134.
Raman, B., C. Pan, G. B. Hurst, M. Rodriguez, Jr., C. K. McKeown, P. K. Lankford, N. F. Samatova and J. R. Mielenz (2009). "Impact of pretreated switchgrass and biomass carbohydrates on Clostridium thermocellum ATCC 27405 cellulosome composition: A quantitative proteomic analysis." PLoS ONE **4**(4): e5271.
Rasmussen, L. E., H. R. Sørensen, J. Vind and A. Viksø-Nielsen (2006). "Mode of action and properties of the beta-xylosidases from Talaromyces emersonii and Trichoderma reesei." Biotechnol Bioeng. **94**(5): 869-876.

Reddy, G. V. B., M. Sridhar and M. H. Gold (2003). "Cleavage of nonphenolic β-1 diarylpropane lignin model dimers by manganese peroxidase from Phanerochaete chrysosporium." European Journal of Biochemistry **270**(2): 284-292.
Reese, E. T., R. G. Siu and H. S. Levinson (1950). "The biological degradation of soluble cellulose derivatives and its relationship to the mechanism of cellulose hydrolysis." J Bacteriol **59**(4): 485-497.
Reetz, M., T., S. Prasad, J. D. Carballeira, Y. Gumulya and M. Bocola (2010b). "Iterative saturation mutagenesis accelerates laboratory evolution of enzyme stereoselectivity: rigorous comparison with traditional methods." Journal of the American Chemical Society **132**(26): 9144-9152.
Reetz, M. T. (2009). "Directed evolution of enantioselective enzymes: an unconventional approach to asymmetric catalysis in organic chemistry." J Org Chem **74**(16): 5767-5778.
Reetz, M. T., P. Soni, L. Fernández, Y. Gumulya and J. D. Carballeira (2010a). "Increasing the stability of an enzyme toward hostile organic solvents by directed evolution based on iterative saturation mutagenesis using the B-FIT method." Chem Commun. **46**(45): 8657-8658.
Reetz, M. T., A. Zonta, K. Schimossek, K.-E. Jaeger and K. Liebeton (1997). "Creation of enantioselective biocatalysts for organic chemistry by in vitro evolution." Angewandte Chemie International Edition in English **36**(24): 2830-2832.
Reinhammar, B. R. M. (1972). "Oxidation-reduction potentials of the electron acceptors in laccases and stellacyanin." Biochimica et Biophysica Acta (BBA) - Bioenergetics **275**(2): 245-259.
Renganathan, V., K. Miki and M. H. Gold (1985). "Multiple molecular forms of diarylpropane oxygenase, an H2O2-requiring, lignin-degrading enzyme from Phanerochaete chrysosporium." Arch Biochem Biophys **241**(1): 304-314.
Renganathan, V., K. Miki and M. H. Gold (1986). "Role of molecular oxygen in lignin peroxidase reactions." Arch Biochem Biophys **246**(1): 155-161.
Reverbel-Leroy, C., S. Pages, A. Belaich, J. P. Belaich and C. Tardif (1997). "The processive endocellulase CelF, a major component of the Clostridium cellulolyticum cellulosome: purification and characterization of the recombinant form." Journal of Bacteriology **179**(1): 46-52.
Rignall, T. R., J. O. Baker, S. L. McCarter, W. S. Adney, T. B. Vinzant, S. R. Decker and M. E. Himmel (2002). Effect of single active-site cleft mutation on product specificity in a thermostable bacterial cellulase. Biotechnology for Fuels and Chemicals. M. Finkelstein, J. McMillan and B. Davison, Humana Press**:** 383-394.
Rodionova, N. A., I. M. Tavobilov and A. M. Bezborodov (1983). "beta-Xylosidase from Aspergillus niger 15: purification and properties." J Appl Biochem **5**(4-5): 300-312.
Rogowski, A., A. Baslé, C. S. Farinas, A. Solovyova, J. C. Mortimer, P. Dupree, H. J. Gilbert and D. N. Bolam (2014). "Evidence that GH115 α-glucuronidase activity, which is required to degrade plant biomass, is dependent on conformational flexibility." J Biol Chem. **289**(1): 53-64.
Roldán, A., V. Palacios, X. Peñate, T. Benítez and L. Pérez (2009). "Use of Trichoderma enzymatic extracts on vinification of Palomino fino grapes in the sherry region." Journal of Food Engineering **75**(3): 375-382.
Romaniec, M. P., U. Fauth, T. Kobayashi, N. S. Huskisson, P. J. Barker and A. L. Demain (1992). "Purification and characterization of a new endoglucanase from Clostridium thermocellum." Biochemical Journal **283**(Pt 1): 69-73.

Roncero, M. B., A. L. Torres, J. F. Colom and T. Vidal (2005). "The effect of xylanase on lignocellulosic components during the bleaching of wood pulps." Bioresour Technol **96**(1): 21-30.
Rouvinen, J., T. Bergfors, T. Teeri, J. K. Knowles and T. A. Jones (1990). "Three-dimensional structure of cellobiohydrolase II from Trichoderma reesei." Science **249**(4967): 380-386.
Rouyi, C., S. Baiya, S. K. Lee, B. Mahong, J. S. Jeon, J. R. Ketudat-Cairns and M. Ketudat-Cairns (2014). "Recombinant expression and characterization of the cytoplasmic rice β-glucosidase Os1BGlu4." PloS one **9**(5): e96712.
Ruelius, H. W., R. M. Kerwin and F. W. Janssen (1968). "Carbohydrate oxidase, a novel enzyme from polyporus obtusus: I. Isolation and purification." Biochim. Biophys. Acta **167**(3): 493-500.
Ruiz-Duenas, F. J., M. J. Martinez and A. T. Martinez (1999). "Molecular characterization of a novel peroxidase isolated from the ligninolytic fungus Pleurotus eryngii." Mol Microbiol **31**(1): 223-235.
Ruiz-Duenas, F. J., M. Morales, E. Garcia, Y. Miki, M. J. Martinez and A. T. Martinez (2009). "Substrate oxidation sites in versatile peroxidase and other basidiomycete peroxidases." J Exp Bot **60**(2): 441-452.
Ruller, R., J. Alponti, L. A. Deliberto, L. M. Zanphorlin, C. B. Machado and R. J. Ward (2014). "Concommitant adaptation of a GH11 xylanase by directed evolution to create an alkali-tolerant/thermophilic enzyme." Protein Engineering Design and Selection **27**(8): 255-262.
Ruscio, J. Z., J. E. Kohn, K. A. Ball and T. Head-Gordon (2009). "The influence of protein dynamics on the success of computational enzyme design." Journal of the American Chemical Society **131**(39): 14111-14115.
Ryabova, O., M. Vršanská, S. Kaneko, W. H. van Zyl and P. Biely (2009). "A novel family of hemicellulolytic α-glucuronidase." FEBS Letters **583**(9): 1457-1462.
Saha, B. C. (2003b). "Purification and properties of an extracellular beta-xylosidase from a newly isolated Fusarium proliferatum." Bioresour Technol. **90**(1): 33-98.
Saha, B. C. and R. J. Bothast (1998). "Purification and characterization of a novel thermostable α-L-arabinofuranosidase from a color-variant strain of Aureobasidium pullulans." Appl Environ Microbiol. **64**(1): 216-220.
Saharay, M., H. Guo and J. C. Smith (2010). "Catalytic mechanism of cellulose degradation by a cellobiohydrolase, CelS." PLoS One **5**(10): e12947.
Sakon, J., D. Irwin, D. B. Wilson and P. A. Karplus (1997). "Structure and mechanism of endo/exocellulase E4 from Thermomonospora fusca." Nat Struct Biol **4**(10): 810-818.
Salamitou, S., M. Lemaire, T. Fujino, H. Ohayon, P. Gounon, P. Beguin and J. P. Aubert (1994). "Subcellular localization of Clostridium thermocellum ORF3p, a protein carrying a receptor for the docking sequence borne by the catalytic components of the cellulosome." J Bacteriol **176**(10): 2828-2834.
Salamitou, S., K. Tokatlidis, P. Béguin and J.-P. Aubert (1992). "Involvement of separate domains of the cellulosomal protein S1 of Clostridium thermocellum in binding to cellulose and in anchoring of catalytic subunits to the cellulosome." FEBS Letters **304**(1): 89-92.
Saloheimo, A., B. Henrissat, A.-M. Hoffrén, O. Teleman and M. Penttilä (1994). "A novel, small endoglucanase gene, egl5, from Trichoderma reesei isolated by expression in yeast." Molecular Microbiology **13**(2): 219-228.

Saloheimo, M., J. Kuja-Panula, E. Ylösmäki, M. Ward and M. Penttilä (2002). "Enzymatic properties and intracellular localization of the novel Trichoderma reesei β-glucosidase BGLII (Cel1A)." Applied and Environmental Microbiology **68**(9): 4546-4553.
Saloheimo, M., P. Lehtovaara, M. Penttilä, T. T. Teeri, J. Ståhlberg, G. Johansson, G. Pettersson, M. Claeyssens, P. Tomme and J. K. C. Knowles (1988). "EGIII, a new endoglucanase from Trichoderma reesei: the characterization of both gene and enzyme." Gene **63**(1): 11-21.
Saloheimo, M., T. Nakari-SetäLä, M. Tenkanen and M. Penttilä (1997). "cDNA cloning of a Trichoderma reesei cellulase and demonstration of endoglucanase activity by expression in yeast." European Journal of Biochemistry **249**(2): 584-591.
Salvachúa, D., A. Prieto, Á. T. Martínez and M. J. Martínez (2013). "Characterization of a novel dye-decolorizing peroxidase (DyP)-type enzyme from Irpex lacteus and its application in enzymatic hydrolysis of wheat straw." Appl Environ Microbiol. **79**(14): 4316-4324
Samejima, M. and K. E. Eriksson (1992). "A comparison of the catalytic properties of cellobiose:quinone oxidoreductase and cellobiose oxidase from Phanerochaete chrysosporium." Eur J Biochem **207**(1): 103-107.
Santos, A., S. Mendes, V. Brissos and L. O. Martins (2014). "New dye-decolorizing peroxidases from Bacillus subtilis and Pseudomonas putida MET94: towards biotechnological applications." Appl Microbiol Biotechnol. **98**(5): 2053-2065.
Satyanarayana, D. V. T. (2013). "Improvement in thermostability of metagenomic GH11 endoxylanase (Mxyl) by site-directed mutagenesis and its applicability in paper pulp bleaching process." Journal of Industrial Microbiology & Biotechnology **40**(12): 1373-1381.
Savile, C. K., J. M. Janey, E. C. Mundorff, J. C. Moore, S. Tam, W. R. Jarvis, J. C. Colbeck, A. Krebber, F. J. Fleitz, J. Brands, P. N. Devine, G. W. Huisman and G. J. Hughes (2010). "Biocatalytic asymmetric synthesis of chiral amines from ketones applied to sitagliptin manufacture." Science **329**(5989): 305-309.
Schaeffer, F., M. Matuschek, G. Guglielmi, I. Miras, P. M. Alzari and P. Béguin (2002). "Duplicated dockerin subdomains of Clostridium thermocellum endoglucanase CelD bind to a cohesin domain of the scaffolding protein CipA with distinct thermodynamic parameters and a negative cooperativity." Biochemistry **41**(7): 2106-2114.
Schaffner, D. W. and R. T. Toledo (1991). "Cellulase production by Trichoderma reesei when cultured on xylose-based media supplemented with sorbose." Biotechnol Bioeng **37**(1): 12-16.
Schimming, S., W. H. Schwarz and W. L. Staudenbauer (1991). "Properties of a thermoactive beta-1,3-1,4-glucanase (lichenase) from Clostridium thermocellum expressed in Escherichia coli." Biochem Biophys Res Commun **177**(1): 447-452.
Schiraldi, C. and M. De Rosa (2002). "The production of biocatalysts and biomolecules from extremophiles." Trends Biotechnol **20**(12): 515-521.
Schneider, P., M. B. Caspersen, K. Mondorf, T. Halkier, L. K. Skov, P. R. Østergaard, K. M. Brown, S. H. Brown and F. Xu (1999). "Characterization of a Coprinus cinereus laccase." Enzyme Microb. Technol. **25**(6-25): 502-508.
Schoemaker, H. E., P. J. Harvey, R. M. Bowen and J. M. Palmer (1985). "On the mechanism of enzymatic fignin breakdown " Febs lett. **183**(1): 7-12.
Schols, H. A., C. C. J. M. Geraeds, M. F. Searle-van Leeuwen, F. J. M. Kormelink and A. G. J. Voragen (1990). "Rhamnogalacturonase: a novel enzyme that degrades the hairy regions of pectins." Carbohydr. Res. **206**(1): 105-115.

Schuster, A. and M. Schmoll (2010). "Biology and biotechnology of Trichoderma." Appl Microbiol Biotechnol **87**(3): 787-799.
Schwarz, W., K. Bronnenmeier and W. L. Staudenbauer (1985). "Molecular cloning of Clostridium thermocellum genes involved in β-glucan degradation in bacteriophage lambda." Biotechnology Letters **7**(12): 859-864
Schwarz, W. H., F. Gräbnitz and W. L. Staudenbauer (1986). "Properties of a Clostridium thermocellum endoglucanase produced in Escherichia coli." Applied and Environmental Microbiology **51**(6): 1293-1299.
Schwarz, W. H., S. Schimming, K. P. Rücknagel, S. Burgschwaiger, G. Kreil and W. L. Staudenbauer (1988). "Nucleotide sequence of the celC gene encoding endoglucanase C of Clostridium thermocellum." Gene **63**(1): 23-30.
Searle-van Leeuwen, M. J. F., L. A. M. Broek, H. A. Schols, G. Beldman and A. G. J. Voragen (1992). "Rhamnogalacturonan acetylesterase: a novel enzyme from Aspergillus aculeatus, specific for the deacetylation of hairy (ramified) regions of pectins." Applied Microbiology and Biotechnology **38**(3): 347-349.
Seegmiller, C. G. and E. F. Jansen (1952). "Polymethylgalacturonase an enzyme causing the glycosidic hydrolysis of esterified pectic substances." J Biol Chem. **195**(1): 327-333.
Seelig, B. (2011). "mRNA display for the selection and evolution of enzymes from in vitro-translated protein libraries." Nat Protoc **6**(4): 540-552.
Seiboth, B., L. Hartl, N. Salovuori, K. Lanthaler, G. D. Robson, J. Vehmaanperä, M. E. Penttilä and C. P. Kubicek (2005). "Role of the bga1-encoded extracellular β-galactosidase of Hypocrea jecorina in cellulase induction by lactose." Applied and Environmental Microbiology **71**(2): 851-857.
Selby, K. and C. C. Maitland (1967). "The cellulase of *Trichoderma viride.* Separation of the components involved in the solubilization of cotton." Biochem. J. **104**: 716-724.
Seyedarabi, A., T. T. To, S. Ali, S. Hussain, M. Fries, R. Madsen, M. H. Clausen, S. Teixteira, B. K. and R. W. Pickersgill (2010). "Structural insights into substrate specificity and the anti beta-elimination mechanism of pectate lyase." Biochemistry **49**(3): 539-546.
Shallom, D. and Y. Shoham (2003). "Microbial hemicellulases." Current Opinion in Microbiology **6**(3): 219-228.
Sharma, N., M. Rathore and M. Sharma (2013b). "Microbial pectinase: sources, characterization and applications." Reviews in Environmental Science and Bio/Technology **12**(1): 45-60.
Sheth, K. and J. K. Alexander (1967). "Cellodextrin phosphorylase from Clostridium thermocellum." Biochim. Biophys. Acta **148**(3): 808-810.
Sheth, K. and J. K. Alexander (1969). "Purification and properties of β-1,4-oligoglucan:orthophosphate glucosyltransferase from Clostridium thermocellum." Journal of Biological Chemistry **244**(2): 457-464.
Shevchik, V. E. and N. Hugouvieux-Cotte-Pattat (1997). "Identification of a bacterial pectin acetyl esterase in Erwinia chrysanthemi 3937." Mol Microbiol **24**(6): 1285-1301.
Shi, H., H. Ding, Y. Huang, L. Wang, Y. Zhang, X. Li and F. Wang (2014). "Expression and characterization of a GH43 endo-arabinanase from Thermotoga thermarum." BMC Biotechnology **14**(35).

Shinmyo, A., D. V. Garcia-Martinez and A. L. Demain (1979). "Studies on the extracellular cellulolytic enzyme complex produced by Clostridium thermocellum." J. Appl. Biochem **1**: 202-209.
Shoemaker, S., V. Schweickart, M. Ladner, D. Gelfand, S. Kwok, K. Myambo and M. Innis (1983). "Molecular cloning of exo-cellobiohydrolase I derived from Trichoderma reesei strain L27." Nat Biotech **1**(8): 691-696.
Shoham, Y., R. Lamed and E. A. Bayer (1999). "The cellulosome concept as an efficient microbial strategy for the degradation of insoluble polysaccharides." Trends in microbiology **7**(7): 275-281.
Shoseyov, O., M. Takagi, M. A. Goldstein and R. H. Doi (1992). "Primary sequence analysis of Clostridium cellulovorans cellulose binding protein A." Proc Natl Acad Sci U S A **89**(8): 3483-3487.
Siau, J. W., S. Chee, H. Makhija, C. M. Wai, S. H. Chandra, S. Peter, P. Dröge and F. J. Ghadessy (2015). "Directed evolution of λ integrase activity and specificity by genetic derepression." Protein Eng Des Sel. **28**(7): 211-220.
Sieber, V., C. A. Martinez and F. H. Arnold (2001). "Libraries of hybrid proteins from distantly related sequences." Nat Biotechnol **19**(5): 456-460.
Sih, C. J. and R. H. McBee (1955). "A cellobiose-phosphorylase in Clostridium thermocellum." Proc. Montana Acad. Sci. **15**: 21-22
Simpson, P. J., H. Xie, D. N. Bolam, H. J. Gilbert and M. P. Williamson (2000). "The structural basis for the ligand specificity of family 2 carbohydrate-binding modules." J Biol Chem **275**(52): 41137-41142.
Sims, I. M., D. J. Craik and A. Bacic (1997). "Structural characterisation of galactoglucomannan secreted by suspension-cultured cells of Nicotiana plumbaginifolia." Carbohydr Res **303**(1): 79-92.
Singh, R. N. and V. K. Akimenko (1993). "Isolation of a cellobiohydrolase of Clostridium thermocellum capable of degrading natural crystalline substrates." Biochemical and Biophysical Research Communications **192**(3): 1123-1130.
Singh, R. N. and V. K. Akimenko (1994). "Isolation and characterization of a complex forming hydrophilic endoglucanase of Clostridium thermocellum." Biochem. Mol. Biol. Int **32**(3): 409-417.
Singh, S. K., C. Heng, J. D. Braker, V. J. Chan, C. C. Lee, D. B. Jordan, L. Yuan and K. Wagschal (2014). "Directed evolution of GH43 β-xylosidase XylBH43 thermal stability and L186 saturation mutagenesis." Journal of Industrial Microbiology & Biotechnology **41**(3): 489-498.
Sipos, B., Z. Benkő, D. Dienes, K. Réczey, L. Viikari and M. Siika-aho (2010). "Characterisation of specific activities and hydrolytic properties of cell-wall-degrading enzymes produced by Trichoderma reesei Rut C30 on different carbon sources." Applied Biochemistry and Biotechnology **161**(1-8): 347-364.
Smiley, J. A. and S. J. Benkovic (1994). "Selection of catalytic antibodies for a biosynthetic reaction from a combinatorial cDNA library by complementation of an auxotrophic Escherichia coli: antibodies for orotate decarboxylation." Proceedings of the National Academy of Sciences **91**(18): 8319-8323.

Smith, M. A., A. Rentmeister, C. D. Snow, T. Wu, M. F. Farrow, F. Mingardon and F. H. Arnold (2012). "A diverse set of family 48 bacterial glycoside hydrolase cellulases created by structure-guided recombination." Febs j **279**(24): 4453-4465.
Smith, S. P. and E. A. Bayer (2013). "Insights into cellulosome assembly and dynamics: from dissection to reconstruction of the supramolecular enzyme complex." Current opinion in structural biology **23**(5): 686-694.
Smith, S. P. and E. A. Bayer (2013). "Insights into cellulosome assembly and dynamics: from dissection to reconstruction of the supramolecular enzyme complex." Curr Opin Struct Biol **23**(5).
Snoek, T., M. Picca Nicolino, S. Van den Bremt, S. Mertens, V. Saels, A. Verplaetse, J. Steensels and K. J. Verstrepen (2015). "Large-scale robot-assisted genome shuffling yields industrial Saccharomyces cerevisiae yeasts with increased ethanol tolerance." Biotechnol Biofuels **8**(32).
Solbak, A. I., T. H. Richardson, R. T. McCann, K. A. Kline, F. Bartnek, G. Tomlinson, X. Tan, L. Parra-Gessert, G. J. Frey, M. Podar, P. Luginbühl, K. A. Gray, E. J. Mathur, D. E. Robertson, M. J. Burk, G. P. Hazlewood, J. M. Short and J. Kerovuo (2005). "Discovery of pectin-degrading enzymes and directed evolution of a novel pectate lyase for processing cotton fabric." Journal of Biological Chemistry **280**(10): 9431-9438.
Solms, J. and H. Deuel (1955). "Über den mechanismus der enzymatischen verseifung von pektinstoffen." Helv. Chim. Acta **38**(1): 321-329.
Solomon, E. I., P. Chen, M. Metz, S. K. Lee and A. E. Palmer (2001). "Oxygen binding, activation, and reduction to water by copper proteins." Angew Chem Int Ed Engl. **40**(24): 4570-4590.
Solomon, E. I., U. M. Sundaram and T. E. Machonkin (1996). "Multicopper oxidases and oxygenases." Chem. Rev. **96**(7): 2563-2605.
Song, L., S. Laguerre, C. Dumon, S. Bozonnet and M. J. O'Donohue (2010). "A high-throughput screening system for the evaluation of biomass-hydrolyzing glycoside hydrolases." Bioresour Technol **101**(21): 8237-8243.
Soutschek-Bauer, E. and W. L. Staudenbauer (1987). "Synthesis and secretion of a heat-stable carboxymethylcellulase from Clostridium thermocellum in Bacillus subtilis and Bacillus stearothermophilus." Molecular and General Genetics MGG **208**(3): 537-541.
Špániková, S. and P. Biely (2006). "Glucuronoyl esterase – Novel carbohydrate esterase produced by Schizophyllum commune." FEBS Lett. **580**(19): 4597-4601.
Spinelli, S., H. P. Fierobe, A. Belaich, J. P. Belaich, B. Henrissat and C. Cambillau (2000). "Crystal structure of a cohesin module from Clostridium cellulolyticum: implications for dockerin recognition." J Mol Biol **304**(2): 189-200.
Spinnler, H. E., B. Lavigne and H. Blachere (1986). "Pectinolytic activity of Clostridium thermocellum: Its use for anaerobic fermentation of sugar beet pulp." Applied Microbiology and Biotechnology **23**(6): 434-437.
Srebotnik, E., K. Messner and R. Foisner (1988). "Penetrability of white rot-degraded pine wood by the lignin peroxidase of Phanerochaete chrysosporium." Appl Environ Microbiol **54**(11): 2608-2614.
Srivastava, S., N. Ghosh and G. Pal (2013). Metagenomics: Mining environmental genomes Biotechnology for Environmental Management and Resource Recovery. R. C. Kuhad and A. Singh, Springer India**:** 161-189.

Stahl, S. W., M. A. Nash, D. B. Fried, M. Slutzki, Y. Barak, E. A. Bayer and H. E. Gaub (2012). "Single-molecule dissection of the high-affinity cohesin-dockerin complex." Proc Natl Acad Sci U S A **109**(50): 20431-20436.
Stahlberg, J., G. Johansson and G. Pettersson (1988). "A binding-site-deficient, catalytically active, core protein of endoglucanase III from the culture filtrate of Trichoderma reesei " Eur. J. Biochem. **173**: 179-183
Stahlberg, J., G. Johansson and G. Pettersson (1993). "Trichoderma reesei has no true exo-cellulase: all intact and truncated cellulases produce new reducing end groups on cellulose." Biochim Biophys Acta **1157**(1): 107-113.
Stålbrand, H. (1993). "Purification and characterization of two β-mannanases from Trichoderma reesei." Journal of Biotechnology **29**(3): 229-242.
Stålbrand, H., A. Saloheimo, J. Vehmaanperä, B. Henrissat and M. Penttilä (1995). "Cloning and expression in Saccharomyces cerevisiae of a Trichoderma reesei beta-mannanase gene containing a cellulose binding domain." Applied and Environmental Microbiology **61**(3): 1090-1097.
Starr, M. P. and F. Moran (1962). "Eliminative split of pectic substances by phytopathogenic soft-rot bacteria." Science **135**(3507): 920-921.
Steenbakkers, P. J. M., H. R. Harhangi, M. W. Bosscher, M. M. C. van der Hooft, J. T. Keltjens, C. van der Drift, G. D. Vogels and H. J. M. op den Camp (2003). "beta-Glucosidase in cellulosome of the anaerobic fungus Piromyces sp. strain E2 is a family 3 glycoside hydrolase." Biochem. J. **370**(3): 963-970.
Steenbakkers, P. J. M., X.-L. Li, E. A. Ximenes, J. G. Arts, H. Chen, L. G. Ljungdahl and H. J. M. Op den Camp (2001). "Noncatalytic docking domains of cellulosomes of anaerobic fungi." Journal of Bacteriology **183**(18): 5325-5333.
Steffler, F., J.-K. Guterl and V. Sieber (2013). "Improvement of thermostable aldehyde dehydrogenase by directed evolution for application in Synthetic Cascade Biomanufacturing." Enzyme and Microbial Technology **53**(5): 307-314.
Stemmer, W. P. C. (1994a). "Rapid evolution of a protein in vitro by DNA shuffling." Nature **370**(6488): 389-391.
Stemmer, W. P. C. (1994b). "DNA shuffling by random fragmentation and reassembly: in vitro recombination for molecular evolution." Proceedings of the National Academy of Sciences **91**(22): 10747-10751.
Stricker, A. R., R. L. Mach and L. H. de Graaff (2008). "Regulation of transcription of cellulases-and hemicellulases-encoding genes in Aspergillus niger and Hypocrea jecorina (Trichoderma reesei)." Appl Microbiol Biotechnol **78**(2): 211-220.
Strobel, H. J., F. C. Caldwell and K. A. Dawson (1995). "Carbohydrate transport by the anaerobic thermophile Clostridium thermocellum LQRI." Applied and Environmental Microbiology **61**(11): 4012-4015.
Stutzenberger, F. (1986). "Hydrolysis products inhibit adsorption of Trichoderma reesei C30 cellulases to protein-extracted lucerne fibres." Enzyme and Microbial Technology **8**(6): 341-344.
Sugano, Y. (2009). "DyP-type peroxidases comprise a novel heme peroxidase family." Cell Mol Life Sci **66**(8): 1387-1403.

Sugano, Y., R. Muramatsu, A. Ichiyanagi, T. Sato and M. Shoda (2007). "DyP, a unique dye-decolorizing peroxidase, represents a novel heme peroxidase family: ASP171 replaces the distal histidine of classical peroxidases." Journal of Biological Chemistry **282**(50): 36652-36658.
Sugano, Y., R. Nakano, K. Sasaki and M. Shoda (2000). "Efficient heterologous expression in Aspergillus oryzae of a unique dye-decolorizing peroxidase, DyP, of Geotrichum candidum Dec 1." Appl. Environ. Microbiol. **66**: 1754-1758.
Sugano, Y., K. Sasaki and M. Shoda (1999). "cDNA cloning and genetic analysis of a novel decolorizing enzyme, peroxidase gene dyp from Geotrichum candidum Dec 1." J.Biosci.Bioeng. **87**(4): 411-417.
Sun, X. F., R. Sun, P. Fowler and M. S. Baird (2005). "Extraction and characterization of original lignin and hemicelluloses from wheat straw." J Agric Food Chem **53**(4): 860-870.
Sunna, A. and G. Antranikian (1997). "Xylanolytic enzymes from fungi and bacteria." Crit Rev Biotechnol **17**(1): 39-67.
Suye, S. (1997). "Purification and properties of alcohol oxidase from Candida methanosorbosa M-2003." Curr Microbiol. **34**(6): 374-377.
Sygmund, C., D. Kracher, S. Scheiblbrandner, K. Zahma, A. K. Felice, W. Harreither, R. Kittl and R. Ludwig (2012). "Characterization of the two Neurospora crassa cellobiose dehydrogenases and their connection to oxidative cellulose degradation." Appl Environ Microbiol **78**(17): 6161-6171.
Takano, M., M. Nakamura and M. Yamaguchi (2010). "Glyoxal oxidase supplies hydrogen peroxide at hyphal tips and on hyphal wall to manganese peroxidase of white-rot fungus Phanerochaete crassa WD1694." Journal of Wood Science **56**(4): 307-313.
Takashima, S., A. Nakamura, M. Hidaka, H. Masaki and T. Uozumi (1999). "Molecular cloning and expression of the novel fungal β-glucosidase genes from Humicola grisea and Trichoderma reesei." Journal of Biochemistry **125**(4): 728-736.
Tamaru, Y. and R. H. Doi (2001). "Pectate lyase A, an enzymatic subunit of the Clostridium cellulovorans cellulosome." Proceedings of the National Academy of Sciences **98**(7): 4125-4129.
Tao, H. and V. W. Cornish (2002). "Milestones in directed enzyme evolution." Curr Opin Chem Biol **6**(6): 858-864.
Taylor, S. V., P. Kast and D. Hilvert (2001). "Investigating and engineering enzymes by genetic selection." Angewandte Chemie International Edition **40**(18): 3310-3335.
Teeri, T., I. Salovuori and J. Knowles (1983). "The molecular cloning of the major cellulase gene from Trichoderma Reesei." Nat Biotech **1**(8): 696-699.
Teeri, T. T., P. Lehtovaara, S. Kauppinen, I. Salovuori and J. Knowles (1987). "Homologous domains in Trichoderma reesei cellulolytic enzymes: Gene sequence and expression of cellobiohydrolase II." Gene **51**(1): 43-52.
Temp, U. and C. Eggert (1999). "Novel interaction between laccase and cellobiose dehydrogenase during pigment synthesis in the white rot fungus Pycnoporus cinnabarinus." Appl. Environ. Microbiol. Mol. Biol. Rev. **65**(2): 389-395.
Tenkanen, M., J. Puls and K. Poutanen (1992). "Two major xylanases of Trichoderma reesei." Enzyme and Microbial Technology **14**(7): 566-574.
Tenkanen, M. and M. Siika-aho (2000). "An alpha-glucuronidase of Schizophyllum commune acting on polymeric xylan." J Biotechnol **78**(2): 149-161.

Tenkanen, M., M. Vršanská, M. Siika-aho, D. W. Wong, V. Puchart, M. Penttilä, M. Saloheimo and P. Biely (2013). "Xylanase XYN IV from Trichoderma reesei showing exo- and endo-xylanase activity." FEBS Journal **280**(1): 285-301.
Teugjas, H. and P. Valjamae (2013). "Product inhibition of cellulases studied with 14C-labeled cellulose substrates." Biotechnol Biofuels **6**(1): 104.
Teze, D., F. Daligault, V. Ferrières, Y. H. Sanejouand and C. Tellier (2015). "Semi-rational approach for converting a GH36 α-glycosidase into an α-transglycosidase." Glycobiology. **25**(4): 420-427.
The Regents of the University of California. (2015). "Trichoderma reesei genome database v2.0." Retrieved July, 16, 2015, from http://genome.jgi-psf.org/Trire2/Trire2.home.html.
Tien, M. and T. K. Kirk (1983). "Lignin-degrading enzyme from the Hymenomycete Phanerochaete chrysosporium burds." Science **221**(4611): 661-663.
Tien, M. and T. K. Kirk (1984). "Lignin-degrading enzyme from Phanerochaete chrysosporium: Purification, characterization, and catalytic properties of a unique H2O2-requiring oxygenase." Proc Natl Acad Sci U S A **81**(8): 2280-2284.
Tilbeurgh, H. V., P. Tomme, M. Claeyssens, R. Bhikhabhai and G. Pettersson (1986). "Limited proteolysis of the cellobiohydrolase I from Trichoderma reesei: Separation of functional domains." Febs lett. **204**(2): 223-227.
Timell, T. E. (1967). "Recent progress in the chemistry of wood hemicelluloses." Wood Science and Technology **1**(1): 45-70.
Tishkov, V. I., A. V. Gusakov, A. S. Cherkashina and A. P. Sinitsyn (2013). "Engineering the pH-optimum of activity of the GH12 family endoglucanase by site-directed mutagenesis." Biochimie **95**(9): 1704-1710.
Tiwari, M. K., R. Singh, R. K. Singh, I. W. Kim and J. K. Lee (2012). "Computational approaches for rational design of proteins with novel functionalities." Comput Struct Biotechnol J. **2**(3): 1-13.
Tokatlidis, K., S. Salamitou, P. Béguin, P. Dhurjati and J.-P. Aubert (1991). "Interaction of the duplicated segment carried by Clostridium thermocellum cellulases with cellulosome components." FEBS Lett. **291**(2): 185-188.
Tokuriki, N. and D. S. Tawfik (2009). "Stability effects of mutations and protein evolvability." Curr Opin Struct Biol. **19**(5): 596-604.
Tomme, P., A. Boraston, B. McLean, J. Kormos, L. Creagh, K. Sturch, N. R. Gilkes, C. A. Haynes, R. A. J. Warren and D. G. Kilburn (1998). "Characterization and affinity applications of cellulose-binding domains." Journal of Chromatography B **715**(1): 283-296.
Tomme, P., H. Van Tilbeurgh, G. Pettersson, J. Van Damme, J. Vandekerckhove, J. Knowles, T. Teeri and M. Claeyssens (1988). "Studies of the cellulolytic system of Trichoderma reesei QM 9414. Analysis of domain function in two cellobiohydrolases by limited proteolysis." European Journal of Biochemistry **170**(3): 575-581.
Tormo, J., R. Lamed, A. J. Chirino, E. Morag, E. A. Bayer, Y. Shoham and T. A. Steitz (1996). "Crystal structure of a bacterial family-III cellulose-binding domain: a general mechanism for attachment to cellulose." EMBO J **15**(21): 5739-5751.
Torronen, A., R. L. Mach, R. Messner, R. Gonzalez, N. Kalkkinen, A. Harkki and C. P. Kubicek (1992). "The two major xylanases from Trichoderma Reesei: characterization of both enzymes and genes." Nat Biotech **10**(11): 1461-1465.

Trojanowski, J., A. Leonowicz and B. Hampel (1966). "Exoenzymes in fungi degrading lignin. II. Demethoxylation of lignin and vanillic acid." Acta Microbiol Pol **15**(1): 17-22.
Trudeau, D. L., T. M. Lee and F. H. Arnold (2014). "Engineered thermostable fungal cellulases exhibit efficient synergistic cellulose hydrolysis at elevated temperatures." Biotechnology and Bioengineering **111**(12): 2390-2397.
Tuka, K., V. V. Zverlov, B. K. Bumazkin, G. A. Velikodvorskaya and A. Y. Strongin (1990). "Cloning and expression of Clostridium thermocellum genes coding for thermostable exoglucanases (cellobiohydrolases) in Escherichia coli cells." Biochemical and Biophysical Research Communications **169**(3): 1055-1060.
Tuka, K., V. V. Zverlov and G. A. Velikodvorskaya (1992). "Synergism between Clostridium thermocellum cellulases cloned in Escherichia coli." Appl Biochem Biotechnol **37**(2): 201-207.
Tuor, U., H. Wariishi, H. E. Schoemaker and M. H. Gold (1992). "Oxidation of phenolic arylglycerol β-aryl ether lignin model compounds by manganese peroxidase from Phanerochaete chrysosporium: oxidative cleavage of an α-carbonyl model compound." Biochemistry **31**(21): 4986-4995.
Turner, N. J. (2009). "Directed evolution drives the next generation of biocatalysts." Nat Chem Biol **5**(8): 567-573.
Umezawa, T., S. Kawai, S. Yokota and T. Higuchi (1986). "Aromatic ring cleavage of various beta-0-4 lignin model dimers by Phanerochaete chrysosporium." Wood Res **73**: 8-17.
Ustinov, B. B., A. V. Gusakov, A. I. Antonov and A. P. Sinitsyn (2008). "Comparison of properties and mode of action of six secreted xylanases from Chrysosporium lucknowense." Enzyme and Microbial Technology **43**(1): 56-65.
Usui, K., K. Ibata, T. Suzuki and K. Kawai (1999). "XynX, a possible exo-xylanase of Aeromonas caviae ME-1 that produces exclusively xylobiose and xylotetraose from xylan." Biosci Biotechnol Biochem **63**(8): 1346-1352.
Utt, E. A., C. K. Eddy, K. F. Keshav and L. O. Ingram (1991). "Sequencing and expression of the Butyrivibrio fibrisolvens xylB gene encoding a novel bifunctional protein with beta-D-xylosidase and alpha-L-arabinofuranosidase activities." Appl Environ Microbiol **57**(4): 1227-1234.
Uzan, E., P. Nousiainen, V. Balland, J. Sipila, F. Piumi, D. Navarro, M. Asther, E. Record and A. Lomascolo (2010). "High redox potential laccases from the ligninolytic fungi Pycnoporus coccineus and Pycnoporus sanguineus suitable for white biotechnology: from gene cloning to enzyme characterization and applications." J Appl Microbiol. **108**(6): 2199-2213.
Uziie, M., M. Matsuo and T. Yasui (1985). "Possible identity of β-Xylosidase and β-Glucosidase of Chaetomium trilaterale." Agricultural and Biological Chemistry **49**(4): 1167-1173.
Vaaje-Kolstad, G., B. Westereng, S. J. Horn, Z. Liu, H. Zhai, M. Sorlie and V. G. H. Eijsink (2010). "An oxidative enzyme boosting the enzymatic conversion of recalcitrant polysaccharides." Science **330**(6001): 219-222.
Valenzuela, S. V., C. Valls, M. B. Roncero, T. Vidal, P. Diaz and F. I. J. Pastor (2013). "Effectiveness of novel xylanases belonging to different GH families on lignin and hexenuronic acids removal from specialty sisal fibres." Journal of Chemical Technology and Biotechnology **89**(3): 401-406.
van den Brink, J. and R. P. de Vries (2011). "Fungal enzyme sets for plant polysaccharide degradation." Appl Microbiol Biotechnol **91**(6): 1477-1492.

van der Vlugt-Bergmans, C. J., P. J. Meeuwsen, A. G. Voragen and A. J. van Ooyen (2000). "Endo-xylogalacturonan hydrolase, a novel pectinolytic enzyme." Appl Environ Microbiol **66**(1): 36-41.
Van Doorslaer, E., H. Kersters-Hilderson and C. K. De Bruyne (1985). "Hydrolysis of β-d-xylo-oligosaccharides by β-d-xylosidase from Bacillus pumilus." Carbohydrate Research **140**(2): 342-346.
Veitch, N. C. (2004). "Horseradish peroxidase: a modern view of a classical enzyme." Phytochemistry **65**(3): 249-259
Verbruggen, M. A., G. Beldman and A. G. Voragen (1998b). "Enzymic degradation of sorghum glucuronoarabinoxylans leading to tentative structures." Carbohydr Res. **306**(1-2): 275-282.
Verma, O. P., A. Singh, N. Singh and O. Chaudhary (2011). "Isolation, purification and characterization of β-glucosidase from Rauvolfia serpentina." J Chem Eng Process Technol **2**(5): 119.
Viña-Gonzalez, J., D. Gonzalez-Perez, P. Ferreira, A. T. Martinez and M. Alcalde (2015). "Focused directed evolution of aryl-alcohol oxidase in yeast using chimeric signal peptides." Appl Environ Microbiol.
Vitali, J., B. Schick, H. C. M. Kester, J. Visser and F. Jurnak (1998). "The three-dimensional structure of Aspergillus niger pectin lyase B at 1.7-Å resolution." Plant physiol. **116**(1): 69-80.
Vlasenko, E., M. Schülein, J. Cherry and F. Xu (2010). "Substrate specificity of family 5, 6, 7, 9, 12, and 45 endoglucanases." Bioresource Technology **101**(7): 2405-2411.
Vocadlo, D. J. and G. J. Davies (2008). "Mechanistic insights into glycosidase chemistry." Current Opinion in Chemical Biology **12**: 539-555.
Volc, J., P. Sedmera and V. Musílek (1978). "Glucose-2-oxidase activity and accumulation of D-arabino-2-hexosulose in cultures of the basidiomycete Oudemansiella mucida." Folia Microbiol (Praha) **23**(4): 292-298.
von Gal Milanezi, N., D. P. Gómez Mendoza, F. Gonçalves de Siqueira, L. Paulino Silva, C. A. Ornelas Ricart and E. X. Ferreira Filho (2012). "Isolation and characterization of a xylan-degrading enzyme from Aspergillus niger van Tieghem LPM 93 with potential for industrial applications." Bioenerg. Res. **5**(2): 363-371.
Vu, V. H. and K. Kim (2012). "Improvement of cellulase activity using error-prone rolling circle amplification and site-directed mutagenesis." J Microbiol Biotechnol **22**(5): 607-613.
Vu, V. V., W. T. Beeson, E. A. Span, E. R. Farquhar and M. A. Marletta (2014). "A family of starch-active polysaccharide monooxygenases." Proc Natl Acad Sci U S A. **111**(38): 13822-13827.
Wahler, D. and J.-L. Reymond (2001). "Novel methods for biocatalyst screening." Current Opinion in Chemical Biology **5**(2): 152-158.
Wang, C., K. Zhang, C. Zhongjun, H. Cai, W. Honggui and P. Ouyang (2015). "Directed evolution and mutagenesis of lysine decarboxylase from Hafnia alvei AS1.1009 to improve its activity toward efficient cadaverine production." Biotechnology and Bioprocess Engineering **20**(3): 439-446.
Wang, H.-C., Y.-C. Chen and R.-S. Hseu (2014). "Purification and characterization of a cellulolytic multienzyme complex produced by Neocallimastix patriciarum J11." Biochemical and Biophysical Research Communications **451**(2): 190-195.
Wang, J., Q. Zhang, Z. Huang and Z. Liu (2013). "Directed evolution of a family 26 glycoside hydrolase: endo-beta-1,4-mannanase from Pantoea agglomerans A021." J Biotechnol **167**(3): 350-356.

Wang, L., Y. Zhang and P. Gao (2008). "A novel function for the cellulose binding module of cellobiohydrolase I." Sci China C Life Sci **51**(7): 620-629.
Wang, T., X. Liu, Q. Yu, X. Zhang, Y. Qu, P. Gao and T. Wang (2005a). "Directed evolution for engineering pH profile of endoglucanase III from Trichoderma reesei." Biomolecular Engineering **22**(1-3): 89-94.
Wang, W. K., K. Kruus and J. H. Wu (1993). "Cloning and DNA sequence of the gene coding for Clostridium thermocellum cellulase Ss (CelS), a major cellulosome component." J Bacteriol **175**(5): 1293-1302.
Wang, W. K. and J. H. D. Wu (1993). "Structural features of the Clostridium thermocellum cellulase Ss gene." Applied Biochemistry and Biotechnology **39-40**(1): 149-158.
Wang, X. J., Y. J. Peng, L. Q. Zhang, A. N. Li and D. C. Li (2012f). "Directed evolution and structural prediction of cellobiohydrolase II from the thermophilic fungus Chaetomium thermophilum." Appl Microbiol Biotechnol **95**(6): 1469-1478.
Wang, Y., R. Tang, J. Tao, Z. Wang, B. Zheng and Y. Feng (2012e). "Chimeric cellulase matrix for investigating intramolecular synergism between non-hydrolytic disruptive functions of carbohydrate-binding modules and catalytic hydrolysis." J Biol Chem. **287**(35): 29568-29578.
Ward, G., Y. Hadar, I. Bilkis and C. G. Dosoretz (2003). "Mechanistic features of lignin peroxidase-catalyzed oxidation of substituted phenols and 1,2-dimethoxyarenes." J Biol Chem. **278**(41): 39726-39734.
Ward, G., Y. Hadar and C. G. Dosoretz (2001). "Inactivation of lignin peroxidase during oxidation of the highly reactive substrate ferulic acid." Enzyme Microb Technol. **29**(1): 34-41.
Watanabe, A., K. Hiraga, M. Suda, H. Yukawa and M. Inui (2015). "Functional characterization of Corynebacterium alkanolyticum β-xylosidase and xyloside ABC transporter in Corynebacterium glutamicum." Appl Environ Microbiol. .
Watson, B. J., H. Zhang, A. G. Longmire, Y. H. Moon and S. W. Hutcheson (2009). "Processive endoglucanases mediate degradation of cellulose by Saccharophagus degradans." J Bacteriol. **191**(18): 5697-5705.
Wei, Y. D., S. J. Lee, K. S. Lee, Z. Z. Gui, H. J. Yoon, I. Kim, Y. H. Je, X. Guo, H. D. Sohn and B. R. Jin (2005). "N-glycosylation is necessary for enzymatic activity of a beetle (Apriona germari) cellulase." Biochem Biophys Res Commun. **329**(1): 331-336.
Wells, J. A. (1990). "Additivity of mutational effects in proteins." Biochemistry **29**(37): 8509-8517.
Westermark, U. and K.-E. Eriksson (1974a). "Carbohydrate-dependent enzymatic quinone reduction during lignin degradation." Acta Chem. Scand. **28b**(2): 204-208.
Westermark, U. and K.-E. Eriksson (1974b). "Cellobiose-quinone oxidoreductase, a new wood-degrading enzyme from white rot fungi." Acta Chem. Scand. **28b**: 209-214.
Williams, J. S., R. Hoos and S. G. Withers (2000). "Nanomolar versus millimolar inhibition by xylobiose-derived azasugars: significant differences between two structurally distinct xylanases." J Am Chem Soc **122**: 2223-2234.
Williamson, G. (1991). "Purification and characterization of pectin acetylesterase from orange peel." Phytochem. **30**: 445-449.
Williamson, G., C. B. Faulds, J. A. Matthew, D. B. Archer, V. J. Morris, G. J. Brownsey and M. J. Ridout (1990). "Gelation of sugarbeet and citrus pectins using enzymes extracted from orange peel." Carbohydrate Polymers **13**(4): 387-397.

Wilson, C. A. and T. M. Wood (1992). "The anaerobic fungus Neocallimastix frontalis: isolation and properties of a cellulosome-type enzyme fraction with the capacity to solubilize hydrogen-bond-ordered cellulose " Appl Microbiol Biotechnol **37**: 125-129
Wilson, D. B. (2008). Aerobic microbial cellulase systems. Biomass Recalcitrance: Deconstructing the Plant Cell Wall for Bioenergy. M. E. Himmel. Oxford, UK, Blackwell Publishing**:** 374-392.
Wong, D. W. (2009). "Structure and action mechanism of ligninolytic enzymes." Appl Biochem Biotechnol **157**(2): 174-209.
Wood, J. D. and P. M. Wood (1992). "Evidence that cellobiose:quinone oxidoreductase from Phanerochaete chrysosporium is a breakdown product of cellobiose oxidase." Biochim Biophys Acta. **1119**(1): 90-96.
Wood, T. M. and S. I. McCrae (1972). "The purification and properties of the C1 component of Trichoderma koningii cellulase." Biochem. J. **128**(5): 1183-1892.
Wood, T. M. and S. I. McCrae (1978). "The cellulase of Trichoderma koningii. Purification and properties of some endoglucanase components with special reference to their action on cellulose when acting alone and in synergism with the cellobiohydrolase." Biochem J. **171**(1): 61-72.
Wood, T. M. and S. I. McCrae (1979). "Synergism between enzymes involved in the solubilization of native cellulose." Adv Chem Ser **181**: 181-209.
Woodward, J. (1991). "Synergism in cellulase systems." Bioresource Technology **36**(1): 67-75.
Worth, C. L., R. Preissner and T. L. Blundell (2011). "SDM--a server for predicting effects of mutations on protein stability and malfunction." Nucleic Acids Res. **39**(2): W215-222.
Wu, I. and F. H. Arnold (2013). "Engineered thermostable fungal Cel6A and Cel7A cellobiohydrolases hydrolyze cellulose efficiently at elevated temperatures." Biotechnology and Bioengineering **110**(7): 1874-1883.
Xiao, Z., H. Bergeron, S. Grosse, M. Beauchemin, M.-L. Garron, D. Shaya, T. Sulea, M. Cygler and P. C. K. Lau (2008). "Improvement of the thermostability and activity of a pectate lyase by single amino acid substitutions, using a strategy based on melting-temperature-guided sequence alignment." Applied and Environmental Microbiology **74**(4): 1183-1189.
Xiao, Z., X. Zhang, D. J. Gregg and J. N. Saddler (2004). "Effects of sugar inhibition on cellulases and β-glucosidase during enzymatic hydrolysis of softwood substrates." Appl. Biochem. Biotechnol. **113-116**(1-3): 1115-1126.
Xie, H., H. J. Gilbert, S. J. Charnock, G. J. Davies, M. P. Williamson, P. J. Simpson, S. Raghothama, C. M. Fontes, F. M. Dias, L. M. Ferreira and D. N. Bolam (2001). "Clostridium thermocellum Xyn10B carbohydrate-binding module 22-2: the role of conserved amino acids in ligand binding." Biochemistry **40**(31): 9167-9176.
Xiong, H., O. Turunen, O. Pastinen, M. Leisola and N. von Weymarn (2004). "Improved xylanase production by Trichoderma reesei grown on L-arabinose and lactose or D-glucose mixtures." Appl Microbiol Biotechnol **64**(3): 353-358.
Xiong, J.-S., M. Balland-Vanney, Z.-P. Xie, M. Schultze, A. Kondorosi, E. Kondorosi and C. Staehelin (2007). "Molecular cloning of a bifunctional β-xylosidase/α-L-arabinosidase from alfalfa roots: heterologous expression in Medicago truncatula and substrate specificity of the purified enzyme." J. Exp. Bot. **58**(11): 2799-2810.

Xu, F. and H. Ding (2007). "A new kinetic model for heterogeneous (or spatially confined) enzymatic catalysis: contributions from fractal and jamming (overcrowding) effects." Appl Catal A Gen **317**: 70-81.
Xu, H., F. Zhang, H. Shang, X. Li, J. Wang, D. Qiao and Y. Cao (2013a). "Alkalophilic adaptation of XynB endoxylanase from Aspergillus niger via rational design of pKa of catalytic residues." J Biosci Bioeng **115**(6): 618-622.
Xu, J. and J. C. Smith (2010). "Probing the mechanism of cellulosome attachment to the Clostridium thermocellum cell surface: computer simulation of the Type II cohesin-dockerin complex and its variants." Protein Eng Des Sel. **23**(10): 759-768.
Xu, J., N. Takakuwa, M. Nogawa, H. Okada and Y. Morikawa (1998). "A third xylanase from Trichoderma reesei PC-3-7." Applied Microbiology and Biotechnology **49**(6): 718-724.
Xu, M., R. Zhang, X. Liu, J. Shi, Z. Xu and Z. Rao (2013b). "Improving the acidic stability of a β-mannanase from Bacillus subtilis by site-directed mutagenesis." Process Biochemistry **48**(8): 1166-1173.
Yagüe, E., P. Béguin and J. P. Aubert (1990). "Nucleotide sequence and deletion analysis of the cellulase-eneoding gene celH of Clostridium thermocellum " Gene **89**(1): 61-67.
Yamada, R., T. Higo, C. Yoshikawa, H. China, M. Yasuda and H. Ogino (2015). "Random mutagenesis and selection of organic solvent-stable haloperoxidase from Streptomyces aureofaciens." Biotechnol Prog.
Yang, G., J. R. Rich, M. Gilbert, W. W. Wakarchuk, Y. Feng and S. G. Withers (2010). "Fluorescence activated cell sorting as a general ultra-high-throughput screening method for directed evolution of glycosyltransferases." J. Am. Chem. Soc. **132**: 10570-10577.
Yaniv, O., E. Morag, I. Borovok, E. A. Bayer, R. Lamed, F. Frolow and L. J. Shimon (2013). "Structure of a family 3a carbohydrate-binding module from the cellulosomal scaffoldin CipA of Clostridium thermocellum with flanking linkers: implications for cellulosome structure." Acta Crystallogr Sect F Struct Biol Cryst Commun **69**(Pt 7): 733-737.
Yaron, S., E. Morag, E. A. Bayer, R. Lamed and Y. Shoham (1995). "Expression, purification and subunit-binding properties of cohesins 2 and 3 of the Clostridium thermocellum cellulosome." FEBS Letters **360**(2): 121-124.
You, L. and F. H. Arnold (1996). "Directed evolution of subtilisin E in Bacillus subtilis to enhance total activity in aqueous dimethylformamide." Protein Engineering **9**(1): 77-83.
Yu, C.-C., T. Hill, D. H. Kwan, H.-M. Chen, C.-C. Lin, W. Wakarchuk and S. G. Withers (2014). "A plate-based high-throughput activity assay for polysialyltransferase from Neisseria meningitidis." Anal Biochem. **444**: 67-74.
Yuan, K. L. and S. L. Boa (1979). "Purification and characterization of endopolygalacturonase from Rhizopus arrhizus " Journal of Food Science **43**: 721-726.
Zaccolo, M., D. M. Williams, D. M. Brown and E. Gherardi (1996). "An approach to random mutagenesis of DNA using mixtures of triphosphate derivatives of nucleoside analogues." J Mol Biol. **255**(4): 589-603.
Zamocky, M., R. Ludwig, C. Peterbauer, B. M. Hallberg, C. Divne, P. Nicholls and D. Haltrich (2006). "Cellobiose dehydrogenase: a flavocytochrome from wood-degrading, phytopathogenic and saprotropic fungi." Curr Protein Pept Sci **7**(3): 255-280.

Zandleven, J., G. Beldman, M. Bosveld, J. Benen and A. Voragen (2005). "Mode of action of xylogalacturonan hydrolase towards xylogalacturonan and xylogalacturonan oligosaccharides." Biochemical Journal **387**(3): 719-725.
Zeilinger, S., D. Kristufek, I. Arisan-Atac, R. Hodits and C. P. Kubicek (1993). "Conditions of formation, purification, and characterization of an alpha-galactosidase of Trichoderma reesei RUT C-30." Applied and Environmental Microbiology **59**(5): 1347-1353.
Zeng, W., G. Du, J. Chen, J. Li and J. Zhou (2015). "A high-throughput screening procedure for enhancing α-ketoglutaric acid production in Yarrowia lipolytica by random mutagenesis." Process Biochemistry.
Zhang, D., X. Chen, J. Chi, J. Feng, Q. Wu and D. Zhu (2015). "Semi–Rational engineering a carbonyl reductase for the enantioselective reduction of β-amino ketones." ACS Catalysis **5**(4): 2452-2457.
Zhang, M., Z. Jiang, L. Li and P. Katrolia (2009). "Biochemical characterization of a recombinant thermostable β-mannosidase from Thermotoga maritima with transglycosidase activity." J Mol Catal B-Enzym **60**(3-4): 119-124.
Zhang, Q., J. Yang, K. Liang, L. Feng, S. Li, J. Wan, X. Xu, G. Yang, D. Liu and S. Yang (2008). "Binding interaction analysis of the active site and its inhibitors for neuraminidase (N1 subtype) of human influenza virus by the integration of molecular docking, FMO calculation and 3D-QSAR CoMFA modeling." J Chem Inf Model **48**(9): 1802-1812.
Zhang, S., D. C. Irwin and D. B. Wilson (2000). "Site-directed mutation of noncatalytic residues of Thermobifida fusca exocellulase Cel6B." Eur J Biochem **267**(11): 3101-3115.
Zhang, Y. H. P., M. E. Himmel and J. R. Mielenz (2006b). "Outlook for cellulase improvement: screening and selection strategies." Biotechnol Adv **24**(5): 452-481.
Zhao, G., E. Ali, M. Sakka, T. Kimura and K. Sakka (2006a). "Binding of S-layer homology modules from Clostridium thermocellum SdbA to peptidoglycans." Appl Microbiol Biotechnol **70**(4): 464-469.
Zhao, G., H. Li, B. Wamalwa, M. Sakka, T. Kimura and K. Sakka (2006b). "Different binding specificities of S-layer homology modules from Clostridium thermocellum AncA, Slp1, and Slp2." Biosci Biotechnol Biochem **70**(7): 1636-1641.
Zhao, Y., B. Wu, B. Yan and P. Gao (2004). "Mechanism of cellobiose inhibition in cellulose hydrolysis by cellobiohydrolase." Science in China Series C: Life Sciences **47**(1): 18-24.
Zheng, F. and S. Ding (2013). "Processivity and enzymatic mode of a glycoside hydrolase family 5 endoglucanase from Volvariella volvacea." Appl Environ Microbiol **79**(3): 989-996.
Zheng, H., Y. Liu, M. Sun, Y. Han, J. Wang, J. Sun and F. Lu (2014). "Improvement of alkali stability and thermostability of Paenibacillus campinasensis Family-11 xylanase by directed evolution and site-directed mutagenesis." Journal of Industrial Microbiology & Biotechnology **41**(1): 153-162.
Zhou, C., J. Ye, Y. Xue and Y. Ma (2015). "Directed evolution and structural analysis of alkaline pectate lyase from alkaliphilic Bacillus sp. N16-5 for improvement of thermostability for efficient ramie degumming." Appl Environ Microbiol.
Zhou, C., J. Ye, Y. Xue and Y. Ma (2015). "Directed evolution and structural analysis of alkaline pectate lyase from alkaliphilic Bacillus sp. N16-5 for improvement of thermostability for efficient ramie degumming." Appl Environ Microbiol.

Zhou, J., Y.-H. Wang, J. Chu, L.-Z. Luo, Y.-P. Zhuang and S.-L. Zhang (2009). "Optimization of cellulase mixture for efficient hydrolysis of steam-exploded corn stover by statistically designed experiments." Bioresource technology **100**(2): 819-825.
Zong, Z., L. Gao, W. Cai, L. Yu, C. Cui, S. Chen and D. Zhang (2015). "Computer-assisted rational modifications to improve the thermostability of β-glucosidase from Penicillium piceum H16." BioEnergy Research.
Zverlov, V. V., K.-P. Fuchs and W. H. Schwarz (2002b). "Chi18A, the endochitinase in the cellulosome of the thermophilic, cellulolytic bacterium Clostridium thermocellum." Applied and Environmental Microbiology **68**(6): 3176-3179.
Zverlov, V. V., N. Schantz, P. Schmitt-Kopplin and W. H. Schwarz (2005b). "Two new major subunits in the cellulosome of Clostridium thermocellum: xyloglucanase Xgh74A and endoxylanase Xyn10D." Microbiology **151**(10): 3395-3401.
Zverlov, V. V., N. Schantz and W. H. Schwarz (2005a). "A major new component in the cellulosome of Clostridium thermocellum is a processive endo-β-1,4-glucanase producing cellotetraose." FEMS Microbiology Letters **249**(2): 353-358.
Zverlov, V. V., G. A. Velikodvorskaya and W. H. Schwarz (2002a). "A newly described cellulosomal cellobiohydrolase, CelO, from Clostridium thermocellum: investigation of the exo-mode of hydrolysis, and binding capacity to crystalline cellulose." Microbiology **148**(1): 247-255.
Zverlov, V. V., G. A. Velikodvorskaya and W. H. Schwarz (2003). "Two new cellulosome components encoded downstream of celI in the genome of Clostridium thermocellum: the non-processive endoglucanase CelN and the possibly structural protein CseP." Microbiology **149**(2): 515-524.
Zverlov, V. V., G. V. Velikodvorskaya, W. H. Schwarz, K. Bronnenmeier, J. Kellermann and W. L. Staudenbauer (1998). "Multidomain structure and cellulosomal localization of the Clostridium thermocellum cellobiohydrolase CbhA." Journal of Bacteriology **180**(12): 3091-3099.
Zweig, A., W. G. Hodgson and W. H. Jura (1964). "The oxidation of methoxybenzenes " J. Am. Chem. Soc. **86**: 4124-4129.