Recalcitrance of natural plant biomass is a bottleneck for lignocellulose-derived bioethanol production. The current process requires pretreatment to disrupt the plant cell wall structures or remove lignin to provide cellulose more accessible to cellulase, but it is expensive. Plant genetic manipulation is a promising tool to design crops modified lignin as well as glycosyl-hydrolase expression in planta is provided in this short review.

Keywords: bioethanol, cellulase, crop, gene manipulation

Introduction

To date, a shortage of fossil fuel has been become a primary concern that requires rapid, effective solutions. Many researchers, including R&Ds in different companies have been encouraged by governments and private sectors to overcome this problem. The fossil fuel has been estimated to be depleted within 50 years, perhaps in 2050, and the severe economy, social problems, climate changes seem to accelerate fuel consumption. Moreover, the fast growths in industries, economy, transportation and the increasing numbers of populations in Asia, especially in China and India drive demands for substituted, alternative energy. Renewable energies, including heat, electricity and fuel, from biological resources appear as “green” replacement for petroleum-based fuel (Demain, 2009). Bioethanol from lignocellulose is one of the most promising alternatives for large demands on transportation sectors. However, the bioenergy feedstocks seem problematic according to the resistance of microbial degradation that affects the ethanol production process as compared to starch.

From sugars and starch to lignocellulosic bioethanol

In the last three decades, bioethanol produced from sugars and starch has been used as a blend (mixed with gasoline) or a neat in vehicles in Brazil. Regardless the rising prices and the decrease in oil supplies, many countries such as the U.S., European Union (EU) as well as Thailand adopted this platform to substitute the use of gasoline alone. However, the more bioethanol is consumed, the more sugars and starch need to be
produced. As we know sugars and starch is primarily utilized for human as food, thus it will be inadequate for ethanol production and also lead to competitive prices (Demain, 2009). Energy crops have been known as plants grown for biofuel production. The interesting crops that can be used as raw materials are non-food C4, C3 and perennial plants such as sugarcane, switchgrass, poplar and miscanthus, since they give high yield and are easy-to-grown with broad cultivation (Chandel and Singh, 2010). The plant-cell-walls are composed of three major components: cellulose, hemicellulose and lignin. In nature, these polymers are formed into complexed structures, providing recalcitrance for microbial degradation. Cellulose is a homopolymer consisting of D-glucoses linked by β-1,4-glycosidic bonds. Each cellulose chain is connected to other chains by intra- and inter-hydrogen bondings, resulting in tightly-packed microfibrils. The order of crystallinity of cellulose microfibrils is resist to hydrolysis. In contrast, hemicelluloses are heterogenous polymers built up by pentoses (D-xylose, D-arabinose), Hexoses (D-mannose, D-glucose, D-galactose) and sugar acids. Hemicelluloses are branched-polymers, thus easy for enzymes to attack. Lignin is synthesized by a series of oxidation using alcohols, coumaryl, coniferyl and sinapyl alcohols, as precursors to form the phenolic heteropolymer, providing structural supports, water-pressure resistance and waterproofing (Anderson and Akin, 2008).

General process for lignocellulosic ethanol production

The general process for ethanol production from lignocellulose is composed of three main steps; pretreatment, saccharification, and fermentation and distillation. Pretreatment aims to disrupt and/or remove unwanted components in order to make cellulose more accessible to cellulases. The pretreated solids are subsequently hydrolyzed by several enzymes to release soluble sugar monomers, which are required for further fermentation by ethanol-fermentation microorganisms such as *Saccharomyces cerevisiae*. The fermentation broth is concentrated by distillation to desired ethanol concentrations. In practice, the rate limiting step in production process is enzymatic hydrolysis of the pretreated solids (Demain, 2009). Although the pretreatment technology has been developed to make the solids easier to hydrolyze, the severity of pretreatment conditions also lead to other problems such as sugar degradation products, inhibitory compounds, which seriously affect both enzymatic degradation and fermentation. Moreover, the high utility (electricity and water) consumption, waste treatments, and chemical-resisted-bioreactors are required (Margeot et al., 2009). As the crystallinity of cellulose and lignin content in plant-cell-walls are believed to be the major handicaps, development of plant traits with decreased crystallinity and low lignin content would facilitate enzymatic degradation, and pretreatment at mild conditions would be possible to modify the plant-cell-wall components, which would lead to positive economic.

Plant biotechnology: Aids for improving enzymatic degradation

Plant biotechnology especially in the field of gene manipulation is a potential tool for decreasing the recalcitrance of plant biomass. In order to improve cellulose hydrolysis, the reductions of crystallinity and lignin content as well as expression of cellulases and related enzymes in plants are of interest. Reducing crystallinity has been focused based on the hypothesis that reducing crystallinity and increasing more amorphous regions would lead to the higher accessibility of cellulase enzymes to glycan chain. To reach that goal, most researches have relied on disruption by carbohydrate-binding modules (CBMs) and modification of cell-wall proteins (Xie and Peng, 2011). CBM is typically found in carbohydrate-active enzymes such as cellulase and xylanase. It is an additional module that brings the enzyme to the substrate into closed and prolonged contact. Some CBMs such as from *Trichoderma reesei* endoglucanase III and *Cellulomonas fimi* endoglucanase A were found to disrupt crystalline cellulose and prevented flocculation of bacterial cellulose. Introductions of exogenous CBM were found to improve the ruminal digestibility of rice straw, whereas the increase in fiber length, molecular weight of cellulose polymer and a significant decrease in microfibril angle were detected in the CBM-expressed poplar. In addition, a new form of
cellulose fibrils as splayed ribbons was shown instead of the uniform, thin, packed ribbons when cellulose was synthesized in the presence of CBM, and the reduction of crystalline cellulose often coincided with the increase in cellulose biosynthesis. Thus, it was postulated that during cellulose synthesis, CBM slides between each polymerized-glucan, separating the glucan chains. This interference uncouples the cellulose polymerization step from the crystallization step, probably leading to an increased rate of cellulose biosynthesis (Shoseyov et al., 2006).

Modification of cell wall protein also results in reduction of cellulose crystallinity. Cellulose is synthesized at the plasma membrane by a symmetrical rosette of six globular protein complexes, in which each complex contains cellulose synthase (CESA) subunits. In Arabidopsis, there are 10 genes encoding for CESA proteins. CESA1, 3, and 6 are known to require for cellulose biosynthesis during primary cell wall formation, whereas CESA4, 7, and 8 are presumably involved in cellulose deposition in secondary cell wall (Endler and Persson, 2011). It was found that two A. thaliana mutants, isoxaben resistance1-2 and 2-1 (ixr1-2, ixr2-1), with decreased relative crystalline index (RCI) were effectively hydrolyzed by enzymatic degradation. The ixr1-2 and ixr2-1 were mutated by positional cloning at CESA3 and CESA6, respectively. In both cases, the locations for mutation were within C-terminus of each protein in or near a transmembrane spanning domain. The reduced RCI values of the two mutants might be due to alteration of proteins that changed the orientation within CESA membrane complex, leading to abnormal arrangements of glucan chains to growing microfibrils such as irregular angles and disruption of a certain percentage of hydrogen bonding (Harris and DeBolt, 2010). Reducing lignin content will facilitate enzyme accessibility to cellulose. Lignin is known as a physical barrier that causes non-productive binding and inactivation of cellulases. Lignin content can be reduced by down-regulation of genes involved in lignin synthesis (Li et al., 2008). For examples, down-regulation of hydroxycinnamoyl CoA: shikimate hydroxycinnamoyl transferase (HCT) in alfalfa reduced lignin levels and improved saccharification efficiency. The downregulated caffeic acid O-methyltransferase gene in switchgrass showed normal phenotypes with reduced ratio of syringyl:guaiacyl lignin monomer, thereby improving saccharification process with lower enzyme loadings (Fu et al., 2011). However, although reduction of lignin was achieved with improved digestibility and saccharification, the phenotypes of plant traits remain problematic when gene selection is not suitable. For example, the severely reduced 4-coumarate:coenzyme A ligase (4CL) in poplar resulted in irregular vessel and lower growth efficiency, suggesting that adequate lignification is required for the growth. Other abnormal phenotypes such as dwarfing, xylem collapse, mechanical strength and plant fitness have been observed through gene silencing (Abramson et al., 2010). Besides, making lignin more susceptible to hydrolyze by addition of peptides to lignin polymer has been recently proposed. Introduction of the tyrosine-rich peptides to transgenic poplar resulted no changes in lignin content and plant phenotypes. The cross-linking between the peptides and lignin was sensitive to protease digestion, thereby improving lignin removal leading to high sugar release (Liang et al., 2008). Expression of cellulases and related enzymes in plants is another choice for effective break down of lignocellulose. Expression of cellulases in plants would decrease the use of exogenous enzymes, thereby reducing the production cost. Thermophilic cellulases have been considered more superior than mesophilic ones in terms of cell-damage prevention and long-term storage. The thermostable endoglucanases from Thermobifida fusca were successfully expressed in tobacco with regular plant morphology. The expressed-cellulases localized in apoplast showed higher activity than that in cytosol (Jiang et al., 2011). However, since complete cellulose hydrolysis requires synergistic actions of endo- and exo-glucanases and ß-glucosidase, heterogenous expressions of multiple genes, organelle-targeting and expression yields should be considered. Expressions of other cell wall-degrading enzymes, including endoxylanase, feruloyl esterase, acetylesterase have been investigated extensively. As mentioned, cellulose is associated with hemicelluloses and lignin, thereby limiting cellulase accessibility. Hemicellulose is usually cross-linked with lignin through hemicellulose-side chains and cross-linking agents, such as ferulic acid. The expression of fungal ferulic acid esterase in ryegrass rendered the cell wall more accessible for
endoxylanase, whereas the expression of xyloglucan transferase could loosen cell-wall structure (Taylor II et al., 2008), resulting in improved saccharification.

Summary

Saccharification of plant biomass requires pretreatment process and high enzyme loadings that make the entire costs for ethanol production from lignocellulose higher than starch. Gene manipulation appears as a tool to modify plant biomass feedstocks suitable for ethanol production with a viable economy. However, advancement in pretreatment technology, improvement of yeast strains and bioreactor design should not be neglected.

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Literature cited


