Engineering Cell Wall-Degrading Enzymes into Growing Plants to Improve Lignocellulosic Ethanol Production

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The plant cell wall (PCW) represents the most abundant renewable biomass resource for lignocellulosic ethanol production. Economical and efficient degradation of PCW to fermentable sugars is an essential sub-process during lignocellulosic ethanol production. At present, the recalcitrance of PCW to various pretreatments and enzymatic hydrolysis makes the PCW degradation unacceptably expensive. Engineering cell wall-degrading enzymes into growing plants provides a promising solution to lower the PCW degradation cost and increase its degradation efficiency for lignocellulosic ethanol production. Avoiding damage by the expressed biomass-degrading enzymes to growing plants is the key to successful use of this method. Two modern biological technologies can be used to solve this problem. One is to engineer a thermoregulated intein-modified cell wall-degrading enzyme into growing plants. The other is to use the gene-timed expression technique. This editorial will give a brief discussion of opportunities and challenges of engineering cell wall-degrading enzymes into growing plants for improvement of lignocellulosic ethanol production.

Keywords: Cell wall-degrading enzyme; Plant cell wall; Lignocellulosic ethanol production

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Due to its abundance and renewable nature, the plant cell wall (PCW) is regarded as an attractive feedstock for lignocellulosic ethanol production. With ever-increasing energy demands and environmental concerns, lignocellulosic ethanol production from PCW has drawn much attention in recent years (Wang *et al.* 2011; Chen *et al.* 2014). Degradation of PCW to fermentable sugars is an essential sub-process for lignocellulosic ethanol production. Therefore, the economical and efficient degradation of PCW is extremely important in the production of lignocellulosic ethanol (Zhu *et al.* 2016; 2017).

The PCW is a layer of structural material that surrounds the plant cell and extends to the protoplast. It can be divided into primary and secondary cell walls, which are systematically formed during plant cell growth and development. It is mainly composed of cellulose, hemicelluloses, and lignin, as well as minor pectic polysaccharides and wall proteins. Because the PCW has evolved a complex structure and the mechanical strength to resist physical and biochemical digestion in nature, this recalcitrance of PWC to various pretreatments and enzymatic hydrolysis makes its degradation difficult (Wang and Zhu 2010). The degradation of PCW to fermentable sugars generally includes two major processes: pretreatment and enzymatic hydrolysis. The pretreatment often needs to be carried out under the extreme conditions, such as strong acid/base or extreme temperature/pressure, which is costly and may cause environmental pollution. Many efforts have been made to improve pretreatment efficiency and decrease the cost of enzymatic hydrolysis (Zhu *et al.* 2015a,b). To date, the degradation of PCW is still an unacceptably expensive process. It is estimated that the degradation of PCW to fermentable sugars through pretreatment and enzymatic hydrolysis accounts for between one-quarter and one-third of the cost of lignocellulosic ethanol production (Service 2014). Besides the pretreatment, the high cost of the degradation of PCW to fermentable sugars comes from the enzyme consumption during the enzymatic hydrolysis process. The incorporation of cell wall-degrading enzymes into growing plants not only can simplify the pretreatment process but also can decrease the enzyme consumption during the enzymatic hydrolysis process. Therefore, to engineer a cell wall-degrading enzyme into growing plants is considered to be a promising solution to lower the PCW degradation cost for lignocellulosic ethanol production (Wang *et al.* 2016).

Challenges of Engineering Cell Wall-Degrading Enzymes into Growing Plants to Improve Lignocellulosic Ethanol Production

With the development of modern biological technology, it has become easy to engineer a cell wall-degrading enzyme into growing plants. The greatest challenge in such work is to avoid damage to the growth and development of the plants due to the expression of the enzymes. It is also a prerequisite that this method can be successfully used to improve the lignocellulosic ethanol production. Two strategies have been used to solve this problem. One is to engineer a thermoregulated intein-modified cell wall-degrading enzyme into growing plants. Such a system, if successful, holds the expressed enzyme at bay so that it does not damage plants growth and development during their growth phase. After the plants are harvested, they can self-degrade to fermentable sugars under the enzymatic catalysis for lignocellulosic ethanol production through a mild thermo-treatment to activate the degrading enzyme. It is reported that Shen *et al.* have engineered a thermoregulated intein-modified xylanase into maize for consolidated lignocellulosic biomass processing (Hines 2012; Shen *et al.* 2012). The intein-modified xylanase has very low activity and does not damage the growth and development of maize. Processing the harvested maize stover by temperature-regulated xylanase activation and hydrolysis can achieve >90% theoretical glucose and >63% theoretical xylose yields. This strategy has reduced the amount of enzymes required for maize stover pretreatment and hydrolysis during bioprocessing to release fermentable sugars for ethanol production.

The other approach is to use the gene-timed expression technique through chemical- or senescence-inducible promoters. These promoters allow the production of cell wall-degrading enzymes just prior to the plants' harvest, avoiding early production of degrading enzymes that can cause negative impacts on the growth and development of plants. Without any pretreatments, the harvested plants can be auto-hydrolysed to fermentable sugars using the internally generated degrading enzymes for ethanol production. Klose *et al.* (2013) successfully avoided the adverse effects of the expressed mesophilic cellulase in growing plants on their development by using an ethanol-inducible promoter. Furukawa *et al.* (2014) reported that the expressed exo-glucanase in rice under the control of a senescence-inducible promoter does not affect rice growth and development. The harvested rice straw can be auto-hydrolysed to fermentable sugars for ethanol production with high yields without any pretreatments.

As discussed, some progress already has been made in incorporating cell wall-degrading enzymes into growing plants to improve lignocellulosic ethanol production.

Because of the complex nature of the regulation system for expression of the cell wall-degrading enzymes in growing plants, there are still lots of technical problems that need to be solved before this method can come into practical use, while avoiding potential adverse effects on plants' growth and development. More efforts should be made to understand the expression regulation mechanism of the cell wall-degrading enzymes in growing plants and their effects on plants' growth and development. After the envisioned joint efforts, it is reasonable in the future to expect that the incorporation of cell wall-degrading enzymes into growing plants will become an effective method to decrease the PCW degradation cost for lignocellulosic ethanol production.

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