Review

Cellulases for biomass degradation: comparing recombinant cellulase expression platforms

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Improvement of cellulase expression has the potential to change the nature of the biofuel industry. Increasing the economic feasibility of cellulase systems would significantly broaden the range of practicable biomass conversion, lowering the environmental impact of our civilisations' fuel needs. Cellulases are derived from certain fungi and bacteria, which are often difficult to culture on an industrial scale. Accordingly, methods to recombinantly express important cellulases and other glycosyl hydrolase (GH) enzymes are under serious investigation. Herein, we examine the latest developments in bacterial, yeast, plant, and fungal expression systems. We discuss current strategies for producing cellulases, and evaluate the benefits and drawbacks in yield, stability, and activity of enzymes from each system, and the overall progress in the field.

Biofuel production through biomass degradation

The creation of reliable and efficient system processes for biofuel production has gained increasing momentum over the past decade. For the critical step of cellulosic biomass to sugar conversion, research efforts have recently widened scope, exploring synthetic biology techniques (see Glossary) for the production of cellulases and other glycosyl hydrolase (GH) enzymes (Box 1) to enhance or replace native organisms [1]. Efficient cellulase production has developed into a significant bottleneck for the biofuel industry. Although native cellulolytic organisms, particularly fungi like *Trichoderma reesei*, are presently used industrially to produce cellulase mixtures [2], the product yields and titres require improvement for such industrial enzyme-based production systems to be economically viable as the primary fuel source for our future [3]. Currently, heterologous expression techniques fail to even approach the enzyme expression obtained through the 'gold standard' of industrially used *T. reesei* strains. These cellulase mixtures consist predominantly of exoglucanases (cellobiohydrolases), which contribute up to 80% of the total protein, as well as incorporating endoglucanases (up to 15% of the total protein) and lesser amounts of enzymes with other hydrolytic activities [4,5]. The use of *T. reesei* as a cellulase producer is primarily due to its high levels of protein secretion, whereby industrial strains can secrete 40 g/l, and reputedly up to 100 g/l, of extracellular cellulases [4,6]. Despite this, intensive research is underway into novel cellulase producing systems, not only to increase yields and economic feasibility, but also to expand the use of such systems by progressing towards more industrially-flexible bacteria or yeast production systems. Thus, much enzymatic hydrolysis research is aimed at the production of enzymes at concentrations and with activity levels to challenge fungal cellulase production, as well as the use of organisms that would potentially provide new opportunities for changes in the current system processes [3,7].

Owing to the progress in heterologous expression techniques, recombinant enzyme production systems are now promising platforms for efficient industrial cellulase production [1] with many areas where they may enhance the

Glossary

Bacterial cellulosome: cellulosomes, i.e., structures consisting of several GHs attached to a scaffoldin via dockerin–cohesin affinity, which are found in bacterial organisms.
Carbohydrate binding module (CBM): a domain found as part of cellulase enzymes which is responsible for the association of the enzyme to an (often specific) carbohydrate substrate.
Chimeric cellulases: cellulases consisting of a cellulase catalytic domain fused to a second foreign domain, such as a CBM, dockerin, or another catalytic domain via a linker sequence.
Consolidated bioprocessing (CBP) system: a process combining the different key requirements for biomass to biofuel conversion, preferably within a single-step system.
Enzyme yield and activity: the quantity of enzyme produced by a specific organism (as g enzyme/g substrate) and how active that enzyme is against a given cellulosic substrate.
Glycosyl hydrolase (GH): an enzyme that catalyses the hydrolysis of glycosidic bonds, e.g., between two carbohydrates, releasing smaller (sugar) molecules.
Mini-cellulosome: designer cellulosome built out of a small scaffoldin (or other scaffold) to which is attached specific GH enzymes.
Product yield and titre: the amount of biomass converted into desired product (glucose or ethanol) and the total amount of desired product obtainable.
SCHEMA (structure guided protein recombination): computational method that uses protein sequence and structural data of homologous proteins to define amino acid sequence blocks whose recombination most likely leads to functional and stable chimeras.
Synthetic biology: the use of recombinant DNA technologies for the combination of genes and the production of novel proteins.

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Box 1. Cellulases and the GH families

Enzymes hydrolysing the glycosidic bond between carbohydrates or between a carbohydrate and a non-carbohydrate moiety are named glycoside hydrolases (GHs) by the IUBMB. The catalytic mechanism involves two amino acid residues, one as proton donor and the other as nucleophile. To date, 132 GH families organised into 14 clans have been determined (http://www.cazy.org). Cellulase is the term given to the subset of GH enzymes that cleave glycosidic-β-1-4 bonds, for example, those found in cellulose. There are three major types of cellulases: exoglucanases, endoglucanases, and beta-glycosidases [3]. Cellulolytic enzymes are distributed among many GH families with prominent examples for endoglucanases (EC: 3.2.1.4) in families 5, 9, and 12, exoglucanases (EC: 3.2.1.91/176) in families 6 and 7, and β-glucosidases (EC: 3.1.2.21) in families 1 and 3. Some GHs consist of more than one catalytic domain from different families or they are connected with other domains like carbohydrate binding modules (CBMs) for substrate recognition [9,11].

Box 2. Cellulosome systems

A cellulosome is a combination of several tethered cellulolytic enzymes that interrelate and augment each other, enhancing overall cellulase activity [34]. Cellulosomes are built of a scaffoldin backbone that contains several cohesin domains. Each cohesin domain has the capacity to interact with a dockerin domain-carrying enzyme. The final structure is a large complex of cellulase or other GH enzymes, frequently attached onto the cell surface [11]. By the positioning of the enzymes in close proximity, the system acts synergistically to degrade cellulose while enabling cellulose fragments to be collected at high concentrations close to the enzymes, which results in a minimum of negative feedback products [39]. In the presence of a substrate, the cellulosome is normally tagged via one or more CBMs onto the cellulose, and many cellulosomes can modify their composition and activity to fit the substrate [70]. In nature, cellulosomes are found in several anaerobic cellulolytic bacteria, for example, *Clostridium* sp., and are known to play a major role in efficient cellulose degradation by these organisms [8]. Research into the creation of cellulosomes for the production of bioethanol from cellulose has made great progress within recent years (for a review, see [9]).

productivity of biomass to biofuel processing (Figure 1). The past few years have seen significant advances in identifying new cellulases as well as elucidating the structure of cellulases and their mode of action [8–10]. This research has added significantly towards increasing the yield and overall productivity of cellulase enzymes [11]. One approach to achieving economical enzymatic hydrolysis of biomass is to combine GH activity with hydrolysis and fermentation steps in a so-called consolidated bioprocessing (CBP) system [12,13]. However, as yet no single organism has been identified with the requisite properties of cellulosic-based growth under conditions that stimulate substantial ethanol (or alternative biofuel precursor) production [3,11]. Other methods to increase industrial viability of cellulases involve the clustering of cellulase mixtures into highly active forms, such as surface display and/or cellulosome creation (Box 2) and the production of highly adapted cellulases that feature higher yields or activities. One particular target for process improvement includes weakening of the biomass structure itself, either through the use of cellulase auxiliary proteins in pretreatment strategies, or by *in situ* cellulase production within the biomass to be utilised for biofuel production.

This review aims to collate the progress in recombinant cellulase production, with respect to the major advances, benefits, and disadvantages seen with the predominant bacterial, yeast, and plant expression systems (Figure 2), as well as touching on synthetic biological advances in traditional fungal expression hosts and some more exotic systems. In doing so, we will highlight important contributions to the field of recombinant cellulase production and stress where they are of industrial relevance, focusing primarily on the past 3 years.

Bacterial expression systems for cellulase production

Cellulases have been isolated from a number of bacteria, particularly anaerobic species existing in animal digestive systems [3]. Additionally, bacteria such as *Escherichia coli* are commonly used for recombinant enzyme expression. These factors have led to extensive research examining production of cellulases using both established and less common bacterial expression systems.

Recombinant bacterial systems utilised for cellulase production

E. coli remains the most commonly used system for recombinant cellulase protein production, particularly for the

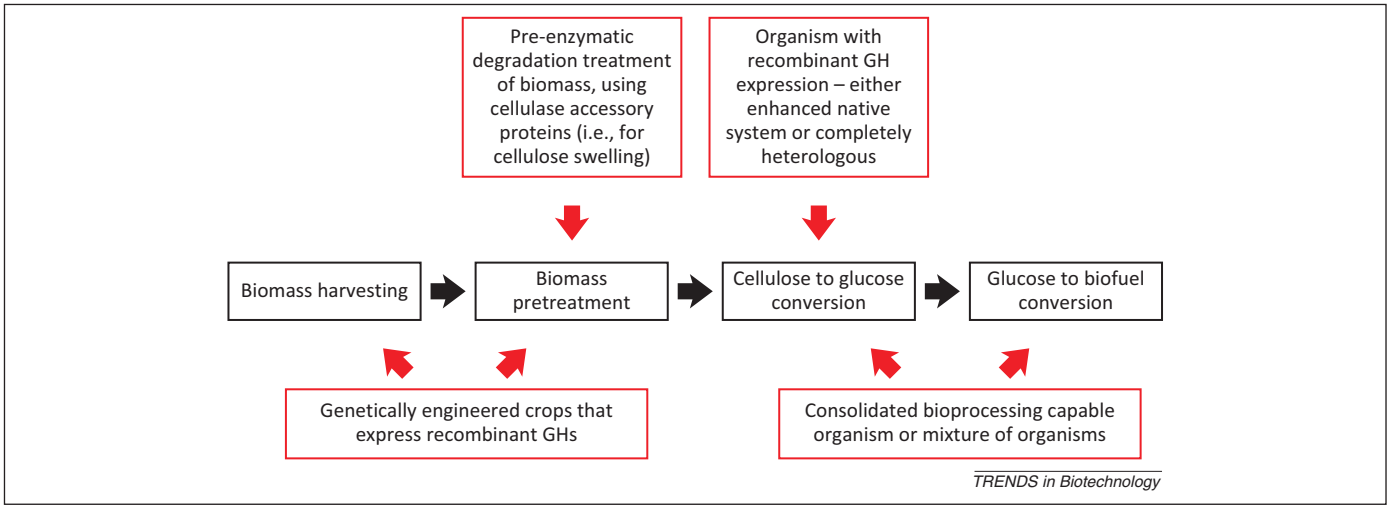

Figure 1. Biofuel production process steps and the areas for potential synthetic biological intervention.