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Revisiting cellulase production and redefining current strategies based on major challenges

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A B S T R A C T

Lignocellulosic biomass has been considered as an important and sustainable source of renewable energy. Cellulose constitutes the major component of the lignocellulosic biomass and also offers maximum recalcitrance towards its fullest utilization. The enzymatic breakdown of cellulose is achieved through cellulases. Diverse forms of microbes including fungi, bacteria, actinomycetes and yeast are known to produce cellulases that have found extensive application in various industries. Due to the current global political unrest over oil prices and the threat of global warming following combustion of fossil fuels, the paradigm of research is now focused on biofuel production from plant biomass. Conventional approaches have not been economically feasible for meeting the demands of the industry. This review provides an update regarding the status of present microbial cellulase production technologies and research with special reference to solid state fermentation and different molecular techniques such as mutagenesis, metabolic engineering and heterologous gene expression of cellulases from different microbial domains with improved catalytic and stability properties. Metagenomic and genomic studies for mining of novel cellulase genes in addition to screening of culturable strains using conventional methods have been advanced. In addition the bottlenecks associated with cellulase production and how the future research needs to be directed to provide a comprehensive technology for the production of cellulases with novel traits for application at an industrial level without economic constraints are discussed.

Contents

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1. Introduction

The idea of converting biomass-derived sugars to biofuels was first proposed in the 1970s [1]. Once again, this idea is being seriously considered as a possible substitute for petroleum-based liquid fuels. Economic and geopolitical factors (high oil prices, environmental concerns, and supply instability) have certainly played a role in reviving an interest in renewable resources. Increasing world-wide demand for energy with unstable and uncertain petroleum sources and concern over global climate change have lead to the resurgence in the development of alternative energy that can displace fossil fuel [2].

The enzymatic conversion of biomass to sugars that can be fermented to ethanol has been conceived of as future source to fuels and chemicals. About 2.9×10^3 million tons of lignocellulosic residues are available from cereal crops and 3×10^3 million tons from pulse and oil seed crops. Also 5.4×10^2 million tons of lignocellulosic biomass is produced annually from plantation crops worldwide [3]. Although abundant in nature, cellulose is a difficult polymer to degrade due to the presence of hydrogen-bonded crystalline fibers. Cellulases hydrolyze the β-1,4-glycosidic linkages of cellulose and produce glucose, cellobiose and cello-oligosaccharides as primary products [4]. The current situation linked with global climate change and a rejuvenated interest in their application in lignocellulose conversion has further stimulated global focus on development of improved cellulase industrial production.

Cellulases have been commercially available for more than 30 years and both basic and applied studies on cellulolytic enzymes have demonstrated their biotechnological potential in various industries including food, animal feed, brewing and wine making, agriculture, biomass refining, pulp and paper, textile and laundry [5,6]. The performance of cellulase mixtures in biomass conversion processes depends on several of their properties including stability, product inhibition, specificity, synergism between the different enzymes, productive binding to the cellulose, physical characteristics as well as the composition of cellulosic biomass [7]

Successful utilization of cellulosic material as a renewable carbon source depends on the development of economically feasible technologies for cellulase production. The primary obstacle impeding the energy production from biomass feedstocks is the general absence of low cost technology for cellulase production. In terms of production technology, low-cost enzymes can be produced by process optimization and genetic engineering of cellulase producing microbial strains. However, the industrial scale-up of this process appears to be still hindered by various technological issues, thus questioning our understanding about the processive and non-processive aspects of the enzyme system and further strengthens the need for an in depth understanding of the enzyme system in terms of its regulation and interaction [8]. A significant portion of research tries to address the bioprocess improvement strategies for enhancing the yield and specific activities of cellulases. Cellulase productivity must be improved further through strain mutation, protoplast fusion, designing of superior biocatalysts by adopting re-engineering techniques and development of recombinant strains for improved cellulase titers. The ideal cellulase complex must be highly active on the intended biomass feedstock, able to completely hydrolyze the biomass, operate well at acidic or alkaline pH, withstand inhibitor stresses and be cost effective. Therefore, unique strategies are required to develop scalable and sustainable enzyme production technology.

The past fifty years have witnessed remarkable progress in (a) isolation of microorganisms producing cellulase (b) improving cellulase production by process optimization (c) purifying and characterizing the cellulase components (d) isolating cellulase from unculturable microbes via metagenomics (f) improving the yield of cellulase by mutation, protoplast fusion, protein engineering and metabolic engineering. This review addresses the conventional methods of cellulase production technologies through bacteria and fungi and further provides an insight to the different novel methods based on our improved understanding of genetic characteristics and its regulation along with advances in state of art technologies in unraveling molecular, proteomic and metabolic functions and their interactions, that has opened the door for designing enzymes with selective traits and desired characteristics involving the cellulase enzyme complex.

2. Cellulose, cellulase and cellulosome

2.1. Cellulose

Over 150 years ago, Anselme Payen, a French chemist, discovered and isolated cellulose from green plants. Cellulose is the most abundant compound in plant cell walls, contributing to about 20–40% of dry weight in the primary cell walls [9]. It generally increases up to 50% in the secondary cell walls, except for a few cases such as cotton seed hair, which consist of 100% cellulose [10].